THE INSTITUTE OF PAPER CHEMISTRY

Appleton, Wisconsin

INCREASED YIELDS IN ALKALINE PULPING. II. A STUDY OF OPTIMUM CONDITIONS FOR USE OF ADDITIVES IN THE PREVENTION OF PEELING

Project 2942

Report Six

A Progress Report

to

MEMBERS OF GROUP PROJECT 2942

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SUMMARY

The stopping reaction was found to occur in cellodextrins, and in cellobiose, when these compounds were heated in 2N NaOH at 75°C and at 90°C. The amount of stopping was of the order of 0.5%, relative to that of peeling. This was determined by acid hydrolysis of the reaction products and determination of the glucose liberated by use of quantitative gas chromatography. It was assumed that the glucose found was derived solely from alkali-resistant glucosidic linkages, and not from unreacted starting material. Gas chromatography of the reaction products before hydrolysis showed no traces of neutral products, such as glucose, cellobiose, or higher oligosaccharides.

It was also found that the yield of isosaccharinic acid, the major product formed in peeling, decreases rapidly with time of heating in alkali at 75°C to a minimum value of 30%; the largest value, 43% at one hour heating, is well below the theoretical value. Also a certain portion of the reaction products is apparently present as a water-insoluble gel. This gel increases in amount with time of alkaline heating. It is originally soluble in the reaction solution, but will not redissolve after drying. Its formation may be related to the disappearance of isosaccharinic acid from the system.

The analyses of the original cellodextrins, cellobiose, acidic products and glucose formed by hydrolysis were done by gas chromatography of the trimethylsilyl derivatives. No acidic products containing a glucosidic bond, presumably formed by the stopping reaction, have been identified on the gas chromatographic curves to date.

Finally, the phenol-sulfuric acid method, used by other workers to determine the extent of stopping, has been found to be invalid. The color developed by this reagent does not represent solely glucose or glucosidic bonds in solution; other products derived by heating glucose with alkali will also react with the reagent.

INTRODUCTION

Originally this project, in its second phase, had a program which included the use of various additives to prevent the peeling reaction. At a meeting of the cooperators in April, 1974 it was decided that the program should concentrate on the stopping reaction, and that no further work should be done on the use of additives. Also, it was decided that some work should be done on a study of the rate of peeling in sodium polysulfide solutions.

In the present report, work is presented on the stopping reaction, occurring in homogeneous solutions of cellobiose and cellodextrins at temperatures of 75 and 90°C. The method of determining the extent of stopping consists of (a) driving the peeling reaction to completion, (b) hydrolyzing the "stopped" solution, and (c) analyzing for glucose liberated in the hydrolysis. This method is quite lengthy in its operation, but is a much more valid means of determining the extent of stopping than the phenol-sulfuric acid method described in Report Five.

The shortcomings of the phenol-sulfuric acid method are also presented in this report. In the preceding report some misgivings were presented about this method and they were confirmed in the present work. This is a very quick and easy method to use, but unfortunately there is a great interference by non-glucosidic products formed in the alkaline reaction and, therefore, no credibility can be attached to the data so obtained.

Future work will be involved with a study of the stopping reaction at temperatures up to 120°C and also the effect of sulfidity on the reaction. An attempt will also be made to isolate glucosyl-metasaccharinic acids, the products

presumably formed in the peeling reaction. Finally, we will explore the effect of sodium polysulfide on the peeling reaction. This will probably involve the formation of oxidation products such as cellobionic acid.

THE STOPPING REACTION

BACKGROUND

The major reaction occurring in the alkaline degradation of oligo- and polysaccharides is peeling, the rapid splitting off of end groups as isosaccharinic acid. This is shown in Fig. 1a and 1b. If the peeling reaction is carried to completion, the final unit, glucose, is isomerized to glucometasaccharinic acid (Fig. 1c). Thus, one would expect a large amount of isosaccharinic acid and a small amount of the meta acid when n is large. This is shown in Fig. 2, a gas chromatographic curve for products from a cellodextrin, where n = 6. The large peak for isosaccharinic acid, has a retention time of about 750 seconds; the smaller peak at 900 seconds, is the glucometasaccharinic acid. The multiple character of the peaks is probably due to the presence of α- and β-isomers, and also to the presence of a mixture of straight chain acids and lactones[1].

In cellobiose, where n = 1, one would expect equal amounts of the iso- and meta-acids, and the chromatographic curve (Fig. 3) bears this out. Finally, the absence of any isosaccharinic acid is shown in Fig. 4, a chromatographic curve for glucose.

The minor reaction occurring in the alkaline degradation of oligosaccharides and polysaccharides is the stopping reaction, the rearrangement of the end unit to a metasaccharinic acid, without the breakage of a glucosidic bond (Fig. 1d). Such units are relatively stable to further alkaline attack; there is no peeling reaction for these compounds, and the glucosidic bond is broken very slowly by alkali or more rapidly by acid hydrolysis (Fig. 1e). Such a stopping

[1]The α- and β-isomers differ in the optical configuration of the carbon atom adjacent to the carboxyl group; this can be seen in Fig. 1a and 1c.

(a) $\xrightarrow{-OH}$ Isosaccharinic Acid + $\mathrm{HC\text{-}OGl}_{n-1}$

(b) $\xrightarrow{-OH}$ n moles of isosaccharinic acid + Glucose

(c) $\xrightarrow{-OH}$ Gluco-metasaccharinic Acid

(d) $\xrightarrow{-OH}$ Glucosyl-metasaccharinic Acid (GMS) (n = 1)
or Cellodextrin-metasaccharinic acid (DMS) (n > 1)

(e) $\xrightarrow{H_3O^+}$ + n moles of glucose

Figure 1. Reactions Occurring in the Peeling and Stopping Reaction of an Oligosaccharide; also Hydrolysis of GMS

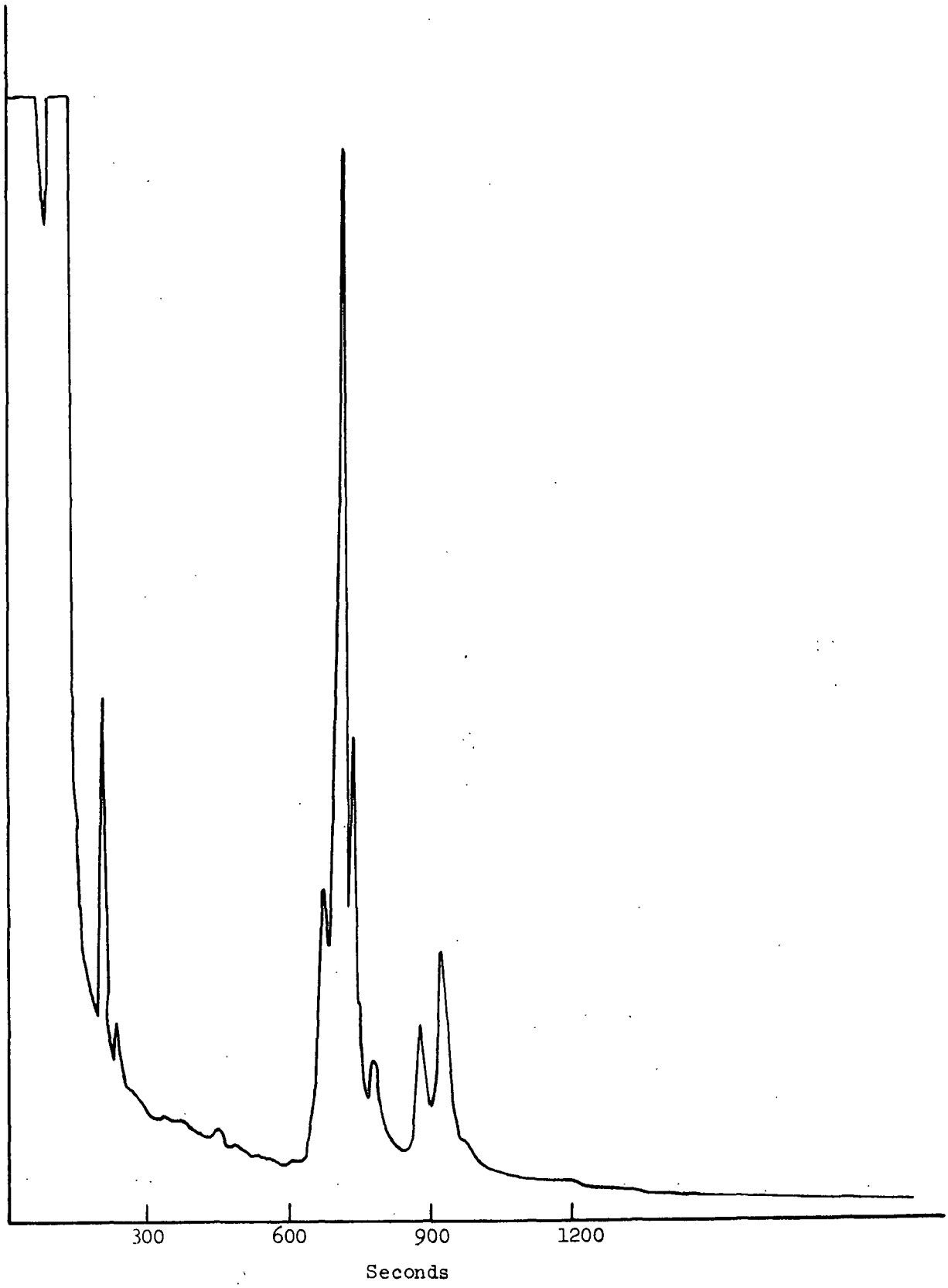

Figure 2. Degradation Products from Cellodextrin, 1 hour in 2N NaOH at 90°C, Showing Predominance of Isosaccharinic Acid. Trimethylsilyl Derivatives on OV-17 Column, 6 ft x 1/8 Inch, Programmed 130 to 200°C, at 2°/minute

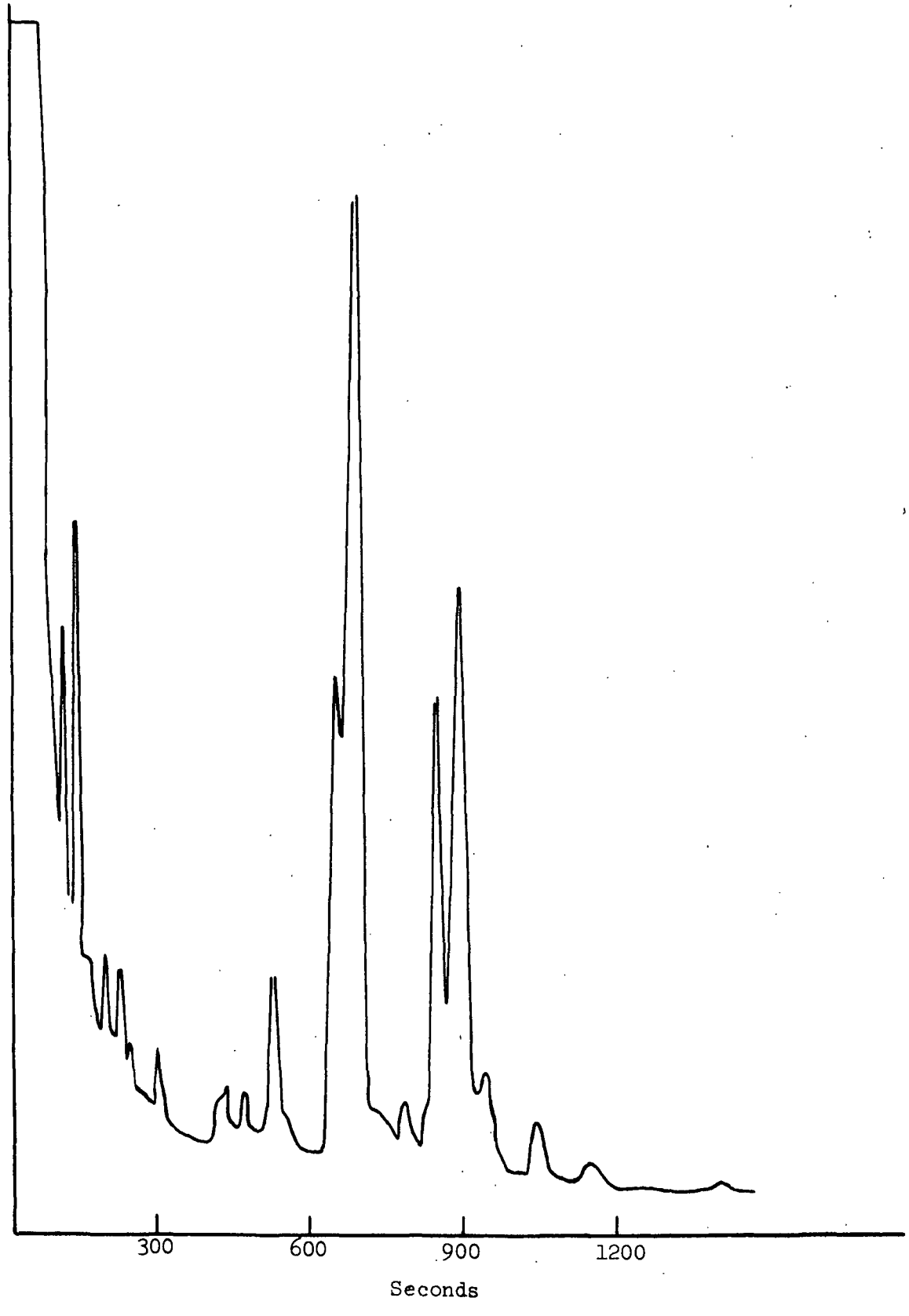

Figure 3. Degradation Products of Cellobiose After 1 hour in 2N NaOH at 75°C; Gas Chromatographic Curve on OV-17 Column, 6 ft x 1/8 Inch, Programmed 130 to 200°C, at 2°/minute

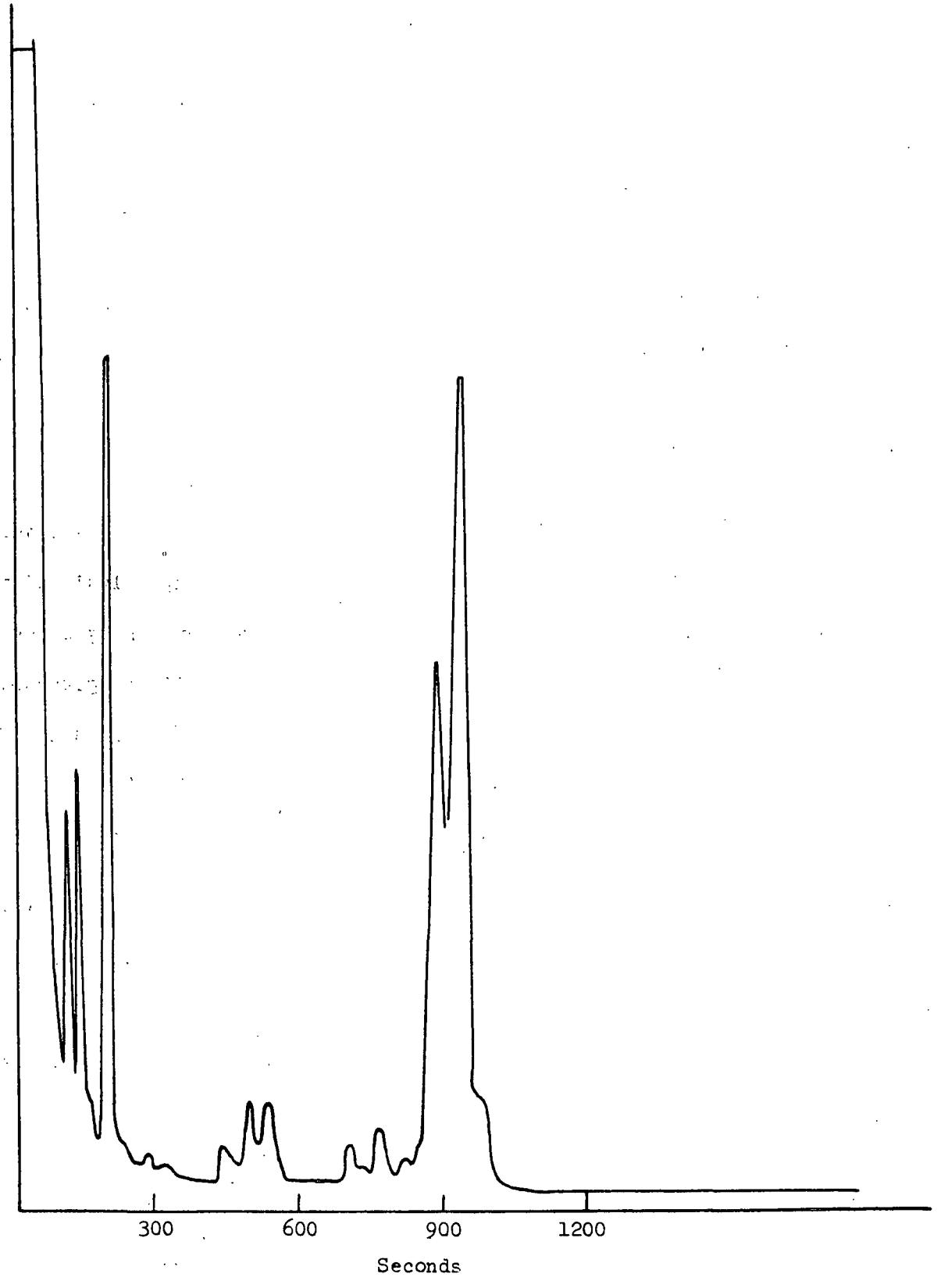

Figure 4. Degradation Products of Glucose; 1 hour in 2N NaOH at 90°C, Gas Chromatographic Curve; OV-17 Column, 6 ft x 1/8 Inch, Programmed 130 to 200°C and 2°/minute. Trimethylsilyl Derivatives

reaction will then lead to the formation of alkali-stable polysaccharides, a desirable product in pulping reactions.

The acid hydrolysis of such a glucosyl-metasaccharinic acid[2] (GMS) or a cellodextrin-metasaccharinic acid (DMS) (Fig. 1e) will give one mole of meta-acid and n moles of glucose. If the peeling reaction is carried to completion, the accompanying stopping reaction can be identified by the liberation of glucose by such an acid hydrolysis. The reaction mixture will then consist of a small amount of glucose and a large amount of various saccharinic acids. Such a system is shown in Fig. 5, where the α-glucose peak is fused into the two peaks representing the gluco-metasaccharinic acid[3].

An analytical scheme for the stopping reaction then involves several steps: (1) carrying the peeling reaction to completion, (2) hydrolysis of the small amount of GMS or DMS present, (3) separation of the resulting glucose from the various saccharinic acids, and (4) quantitative analysis of the glucose. This we have done in this project, with the aid of ion-exchange resins in Step (3) and gas chromatography of the trimethylsilyl derivatives in Step (4). The details of this method are described more fully later in this report.

Earlier workers (1) in this field tried to measure the extent of peeling by the use of a colorimetric method with phenol-sulfuric acid as a reagent (the PSA method). We have found that such a method is highly unsatisfactory, as a spurious color is produced by nonglucose constituents in the alkaline reaction

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[2]This GMS, a C_{12} acid containing a glucosidic bond, should not be confused by the reader with gluco-metasaccharinic acid, a C_6 acid. Please refer to Fig. 1d and 1e for the formulae for the two compounds.

[3]Trimethylsilylation of glucose, where the latter is obtained by concentration of an aqueous solution, will give two peaks, of roughly equal size, for the two anomers, α- and β-glucose.

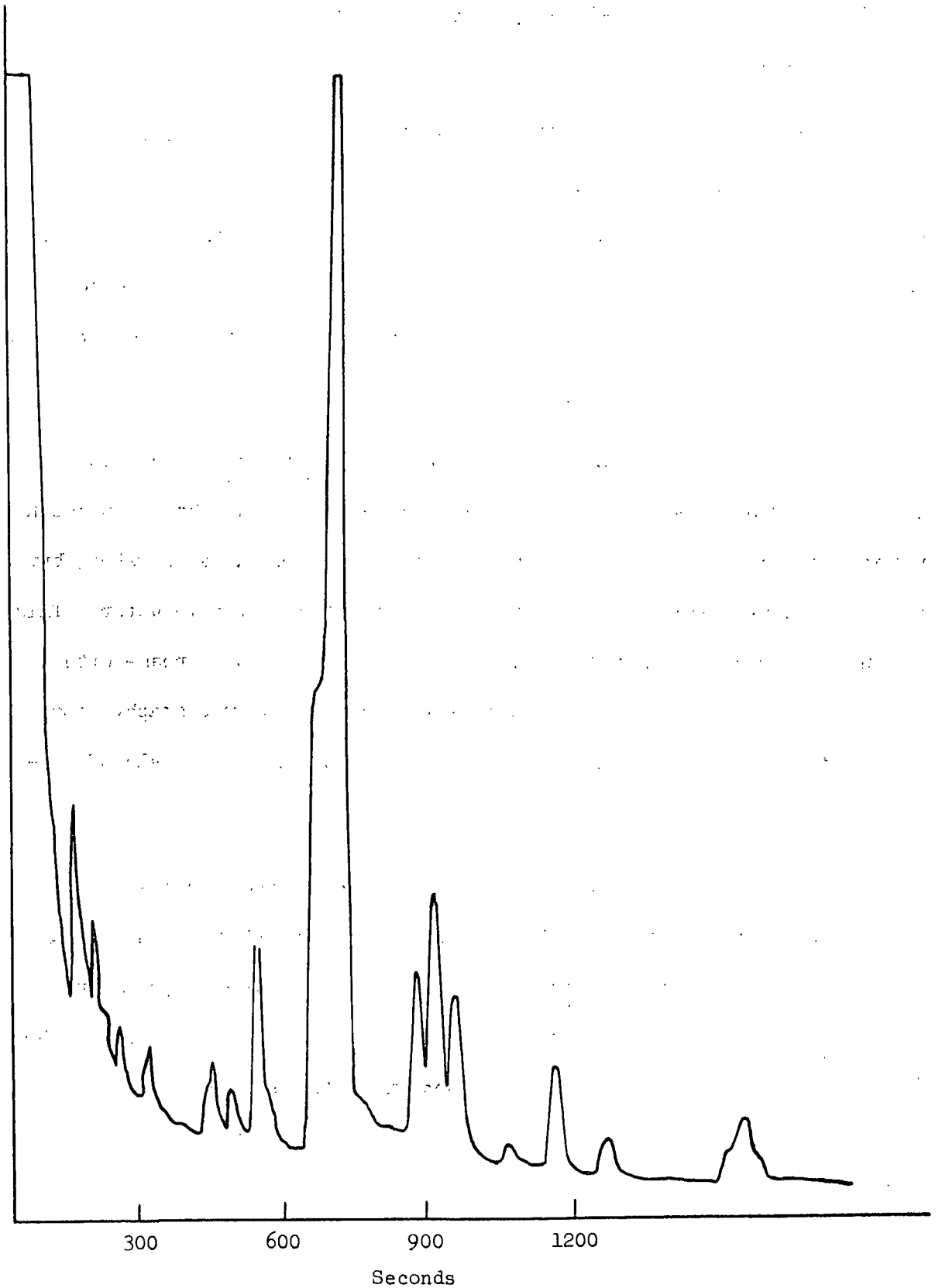

Figure 5. Hydrolyzate of Cellodextrin Reaction Products, Showing Glucose Proximity to Metasaccharinic Acid Peaks. Gas Chromatographic Curve of Trimethylsilyl Derivatives, on OV-17 Column, 6 ft x 1/8 Inch, 130 to 200°C, at 2°/minute

mixtures. Originally it was thought to be a very good method in that it quickly hydrolyzed the glucosidic bonds present and gave a color proportional to the amount of glucose released.

The reactions presented in Fig. 1a to 1d are the major reactions occurring in an alkaline system. However, fragmentation of sugar units may occur, and many small peaks appear in the gas chromatographic curves. This fragmentation occurs especially in the degradation of glucose, to give products such as lactic acid, a C_3-acid, and C_4- and C_5-metasaccharinic acids. Peaks representing such products are shown in Fig. 6.

In addition to these fragmentation reactions, aldol condensations can occur, the combining of fragments to give larger molecules. This has been shown presumably in the formation of a "gel" which is originally soluble in water, but after concentration of a solution to dryness, will not redissolve in water. This gel formation is characterized by three facts: (1) it seems to increase with time of alkaline heating, (2) attempted silylation and gas chromatography show no products, and (3) the gel formation may be related to a loss of yield of isosaccharinic acid.

In the present work we have used primarily an oligosaccharide, a cellodextrin mixture with an average DP = 7. The advantage of such a starting material is that the resulting DMS, when hydrolyzed, will give a large amount of glucose, in contrast to the GMS derived from cellobiose. The analysis of the latter system is more difficult, a matter of analyzing for a "needle in a haystack."

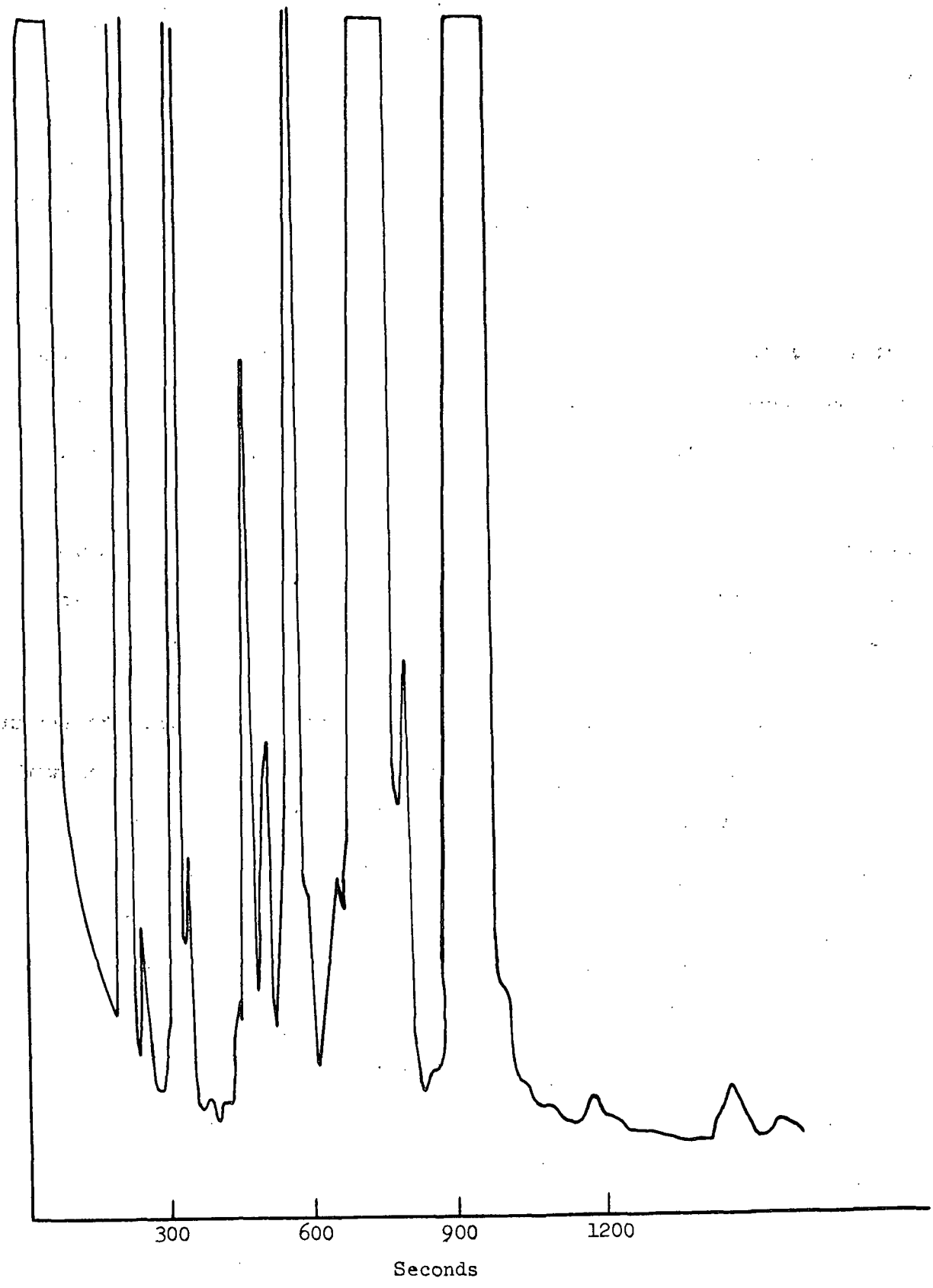

Figure 6. Degradation Products from Cellodextrin, 1 hour in 2N NaOH at 90°C, Gas Chromatographic Curve at High Sensitivity, Trimethylsilyl Derivatives on OV-17 Column, 6 ft x 1/8 Inch, 130 to 200°C, at 2°/minute

DISCUSSION OF EXPERIMENTAL RESULTS

THE PHENOL-SULFURIC ACID METHOD

A conflict exists in the literature about the extent of the stopping reaction, relative to that of the peeling reaction. Lindberg, et al. (1) treated various disaccharides with alkali for extended periods of time and found a certain amount of "residual absorbance" when the reaction mixture was treated with a phenol-sulfuric acid reagent. The absorbance was attributed to glucose bound up with a metasaccharinic acid; this GMS was stable to alkali but hydrolyzed by the acidic PSA reagent to give first free glucose and then a color, measured at 490 nm. The values these workers obtained ranged from 2 to 12% stopping reaction. These values are very high compared with the data obtained by Lai and Sarkanen (2) for the stopping reaction with polysaccharides; these latter workers obtained values of the order of 0.3%.

In this project we have found that the PSA method is invalid for measuring the amount of glucose remaining in a solution after extensive treatment with alkali. First we noted that this "residual absorbance," obtained from the alkaline degradation products of cellobiose, differs from the normal PSA absorbance obtained with pure glucose. The latter shows a maximum at 490 nm only, and the former shows two peaks, a small one at 490 nm and a large one at 415 nm. Secondly, this same residual absorbance was obtained by treating glucose, as well as cellobiose, with alkali and then subjecting the solution to a PSA treatment. Since glucose contains no glucosidic bond, this residual absorbance cannot be attributed to an alkali-stable glucosidic bond.

THE ACID HYDROLYSIS METHOD

We reasoned that a different approach was needed, another method of determining the presence of a glucosidic bond. So we hydrolyzed reaction mixtures with 1N sulfuric acid at 90°C, and then separated the resulting glucose from the saccharinic acids by use of a mixed bed resin. As shown in Fig. 5, the α-glucose peak of the gas chromatograph is fused with two peaks from gluco-metasaccharinic acid. After removal of the acids and lactones, the glucose peaks could be determined quantitatively by comparison of the peak areas with those of an internal standard, inositol.

The amount of glucose thus analyzed for amounted to between 1 and 2%, based on the original cellodextrin. The amounts found did not show a trend with time of heating in alkali, but appeared to show a random scatter, based on the limitations of the analysis. The largest variable was probably the retention of some glucose on the mixed bed resin (see Table I).

The glucose isolated was originally bound to gluco-metasaccharinic acid, and the size of the DMS concerned will vary from a DP of 7 (6 glucose units) to a DP of 2 (one glucose unit). Since the DMS is being formed continuously from a cellodextrin of constantly decreased DP, from DP = 7 originally to a final DP of 2, the average DMS will have a DP of 3 to 4. Using this number as divisor into the percentage glucose found, will give the average amount of DMS formed, or the amount of metasaccharinic acid formed in the stopping reaction. The resultant value ranges from a value of 0.25% stopping (1.0% divided by 4) to a maximum of 0.77% (2.3% divided by 3). These values are of the order found by Lai and Sarkanen (2) and do not agree at all with the data reported by Lindberg, et al. (1).

TABLE I

YIELD OF GLUCOSE FROM HYDROLYSIS OF ALKALI-DEGRADED OLIGOSACCHARIDES

DP of Starting Material	Temp., °C	Time, hr	Gl/In Ratio	Conversion Factor	Glucose Yield, based on starting material, %	Glucose Yield, based on peeling reaction, %
7	75	1	0.068	30	2.0	0.67
		2	0.078	30	2.3	0.77
		4	0.032	30	0.96	0.32
		16	0.055	30	1.65	0.55
7	90	4	0.112	10	1.12	0.37
2	90	4	0.019	10	0.19	0.19

Note — the conversion factors are (a) 30, based on a 1/3 recovery of glucose (Gl) from MB-3 resin, and a 1/10 ratio of inositol (In) to the original oligosaccharide; and (b) 10, for a second series of experiments where more complete washing of the MB-3 resin gave almost complete recovery of glucose.

The yield of glucose, based on peeling, uses a value of DP = 4 for the average DP of the cellodextrin during the degradations, starting with DP = 7. In the case of cellobiose, DP = 2, the nature of the starting material that peels is constant.

Originally we had tried to relate the amount of glucose formed to the amount of isosaccharinic acid, but the low yields of the latter acid prevented its use as a measure of the amount of peeling. Therefore, we have based the glucose yield on the original cellodextrin.

The one experiment with cellobiose (4 hours at 90°C) shows a smaller amount of GMS formed by stopping, than did the several experiments with cellodextrins. Ideally, the extent of stopping, relative to peeling, should be the same in all cases. The analytical data given in Table I show a lot of scatter. This may be due to the many operations involved, hydrolysis, resin treatments,

conversion to trimethylsilyl derivatives, and volatilization of the chromatographic column. The two most probable sources of error, are absorption of some glucose on the MB-3 column, despite extensive washing, and absorption of small amounts of the trimethylsilyl glucose on the chromatographic column. In the cases where smaller amounts of glucose are present, as in the cellobiose experiment, these two factors would lead to smaller amounts of material in the final analysis.

As mentioned in Report Five, Rowell, et al. (3) were unable to isolate GIS from cellobiose and concluded that the stopping reaction did not occur with cellobiose, in contrast to the behavior of cellulose. We were of that opinion originally, also, and so tried out the cellodextrins; these compounds with more glucose units than cellobiose, would be better model compounds for cellulose, and therefore more likely to give the stopping reaction. Apparently, it is a matter of looking for a small fragment, and that derived from cellobiose is very small, and can be easily overlooked. The glucose we found after hydrolysis was very small; the ratio of the glucose to inositol peak areas was about 1 to 50, and the relative areas were determined by use of a digital integrator.

RELATION OF ISOSACCHARINIC ACID TO PEELING

Originally we had tried to relate the amount of glucose formed to the amount of isosaccharinic acid, but the low yields of the latter acid prevented its use as a measure of the amount of peeling. Therefore, we have based the glucose yield on the original cellodextrin or cellobiose.

The yields of isosaccharinic acid (Table II) are much lower than predicted, and fall off rapidly in the first four hours of alkaline heating.

The highest value found, 45.8%, is much lower than that predicted. The stoichiometric yield of the iso acid, if all the 6 glucose units are split off as such, would be of the order of 85%, if the acid is measured as the lactone. Such low yields have been noted in pulping reactions, and found to decrease with increasing temperature (4).

TABLE II

YIELD OF ISOSACCHARINIC ACID FROM ALKALI-DEGRADED OLIGOSACCHARIDES

DP of Starting Material	Temp., °C	Time, hr	Acid/Inositol Ratio	Conversion Factor	Acid, based on starting material, %
7	75	1	2.29	20	45.8
		2	1.61	20	32.4
		4	1.54	20	30.8
		16	1.50	20	30.0
7	90	4	1.60	20	32.0
2	90	4	1.08	20	21.6

Note — Conversion factor is based on 1/10 ratio of inositol to starting material, and a 2X response factor for inositol to the acid in the gas chromatographic analysis.

The above values may be low by a factor of up to 25%, as only the lactone peak area was measured on the gas chromatograph. The acid peak, which is present in only a small amount, has the same retention time as the gluco-metasaccharinic acid peak, and so cannot be measured. An acid solution (pH 3) is used for derivatization, and contains both lactone and acid. Derivatization of the sodium salts from an alkaline solution (pH 10) will give only a peak, on the chromatograph, for the acid. However, the salts are only slightly soluble in the trimethylsilylation reagent, and we have found it easier to work with the acidic solutions.

PRESENCE OF AN ACIDIC PRODUCT FORMED BY STOPPING

We have not been able to find a peak on the gas chromatograph representing a GMS (glucosyl-metasaccharinic acid) or a DMS (cellodextrin-metasaccharinic acid such as G-6-meta acid). Presumably the GMS should be a very small peak in the same region as that of cellobiose. This conclusion is based on the fact that cellobionic acid has a peak in the same region as α- and β-cellobiose. Similarly, the DMS peaks should be in the same region as that of the original cellodextrins.

Several reasons can be presented for the absence of such peaks. First, if the extent of stopping is very small, of the order of 0.3% for cellobiose, the peak for GMS would be extremely small compared with the size of the sample injected. Secondly, the background for a chromatographic column must be free of spurious peaks; we have had some problems with OV-17 columns when the injection chamber is operated at temperatures above 300°C. Finally, a third supposition is that the GMS or the DMS may polymerize as a large molecule and therefore not show up on the GLC in the region of DP 6 or below. Saccharinic acids form lactones readily, and it may be possible that a compound such as GMS or DMS may form an ester bond with another molecule rather than an internal ester or lactone bond. The 4-position in the acid unit is blocked by a glucosyl group, and the dimeric acid GMS may prefer to form an inter-ester bond with another molecule rather than an intra-ester lactone bond at C-5.

NATURE OF THE INSOLUBLE GEL

This product was not investigated in any detail, as our main concern was the stopping reaction. However, we did ascertain several facts about it.

(a) It seems to increase in extent with time of heating in alkali. A solution of dextrin, dissolved in alkali, then worked up without heating, gave no gel at all. Qualitative observations of residues showed greater amounts of gel as the heating period was increased. Quantitative determination of the gel from a cellodextrin, heated in 2N NaOH for 20 hours at 90°C, gave a yield of 32%.

(b) The gel is also formed from cellobiose, as well as from the cellodextrins. This formation of a "polymer" was noted many years ago by J. U. Nef (5), a pioneer in the investigation of saccharinic acids, formed by the action of alkali on simple sugars.

(c) The gel is not hydrolyzed by the action of hot 1N sulfuric acid. It is not soluble in the acid, and analysis of the acid solution by GLC shows only a very small peak in the inositol region, probably due to small amounts of material not washed out of the original gel.

(d) The inertness of the gel to hot acid suggests the presence of carbon-carbon bonds, probably formed by an aldol condensation. Such condensations in alkali lead to the formation of higher sugars (hexoses and pentoses from formaldehyde) and also to the formation of C_6-saccharinic acids from C_5-sugars (6). Ultimately, high-molecular weight material might be formed in this manner.

(e) We do not know the role of such polymer formation in the alkaline cooking of polysaccharides, or whether such a reaction could involve the polysaccharides as well as the soluble constituents in a pulping liquor. Creation of carbon-carbon bonds with a polysaccharide would be similar to the formation of a graft-polymer.

We have noted the loss of inositol, when added as an internal standard at the beginning of an alkaline degradation. For that reason we have added this compound only after the heating period has been terminated and the alkaline solution cooled.

(f) Finally we have a feeling that the formation of the gel, as a water-insoluble residue, is much slower than the decrease in the yield of isosaccharinic acid. This is only a qualitative observation, as we have not attempted to determine quantitatively the increase in yield of the gel with time of heating, nor the yields of isosaccharinic acid at short times of alkaline degradation of cellodextrins. It may be that peeling goes by two pathways (a) splitting off a glucose unit as isosaccharinic acid and (b) splitting off a glucose unit as an unknown reactive intermediate that condenses to a gel. This latter condensation may be for the intermediate alone, or with other compounds present in the system [as saccharinic acids or inositol, cited in (e) above]. The data in Table II are not sufficient to establish the stability of isosaccharinic acid in an alkaline medium, in the presence of a cellodextrin and its various degradation products.

COMPLETENESS OF PEELING OF CELLODEXTRINS

The validity of the glucose found by hydrolysis as a measure of the stopping reaction depends on the absence of any undegraded starting material. In the case of cellobiose we know from the first-order kinetic plot of disappearance of disaccharides in the reaction of cellobiose with 2N sodium hydroxide at 75°C (Report Four, page 26) that the half-life of cellobiose is about 120 seconds. If we assume that such a plot is completely linear, then for a time of 1200 seconds or ten times the half-life, only 0.001 mole of material should be left from 1.000 mole of starting material.

Although this time of 1200 seconds is a time for the "complete" degradation of cellobiose to glucose, the time for the glucose intermediate to reach the value of 0.001 mole will be greater than 1200 seconds. And in the case of a cellodextrin with DP = 7, the time for "complete degradation of the final intermediate, glucose, will be still longer. Therefore, we have constructed a computer program for such a series of consecutive reactions (7) assuming that the rate of breakage of each glucosidic bond in such a cellodextrin is the same. A plot of the change in concentration of the starting material and the several intermediates is shown in Fig. 7. Numerical values for selected time intervals are given in Table III.

From this table, based on complete linearity of a pseudo-unimolecular reaction rate, we can see that the final intermediate, glucose with DP = 1, is down to a concentration of 0.001 mole in about 3030 seconds, or less than 1 hour at 75°C. Similarly, the time for cellobiose to be completely converted to saccharinic acids (or nonglucose units) is 1590 seconds, (using the change in concentrations of DP = 7 and DP = 6 as DP = 2 and DP = 1).

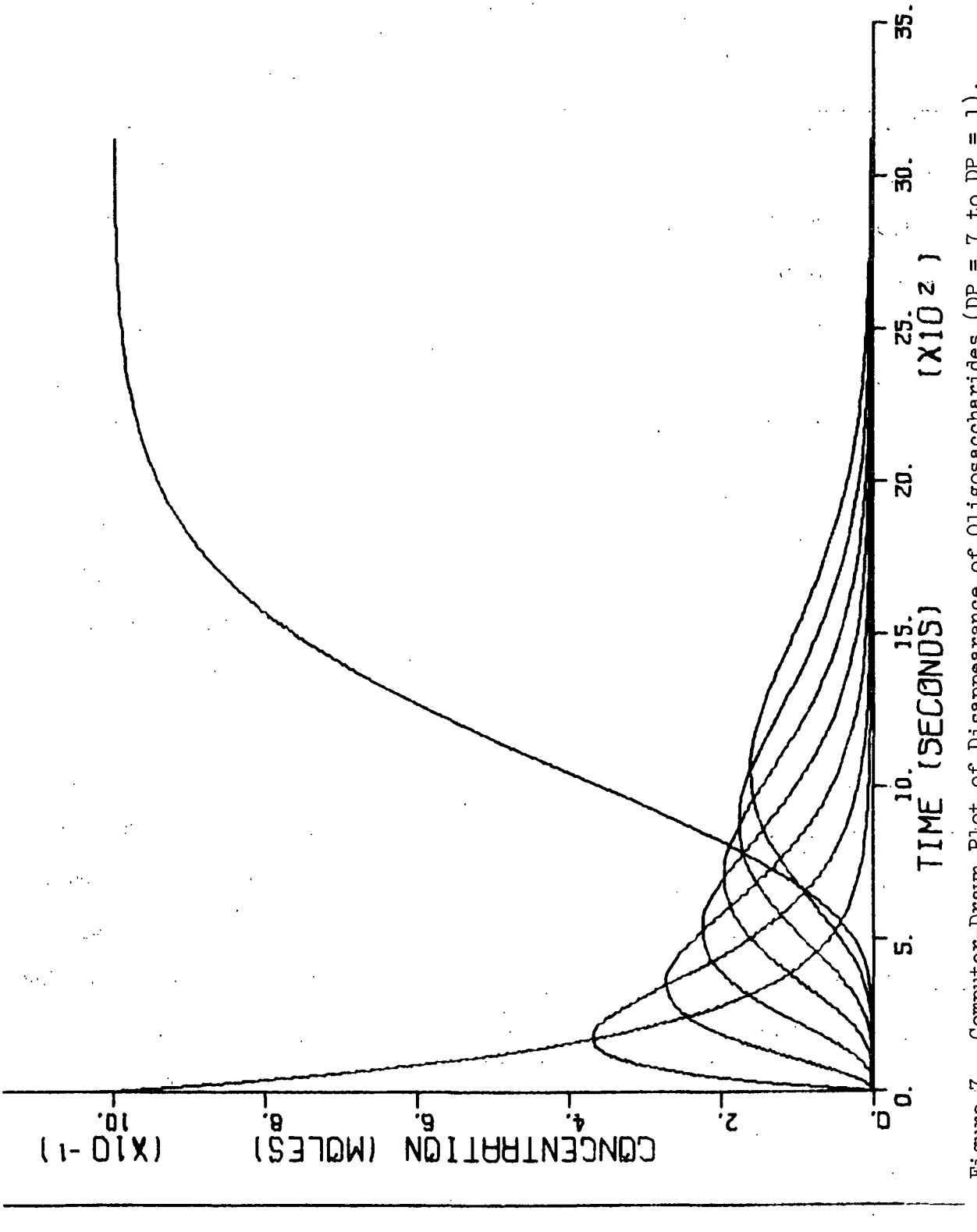

Figure 7. Computer-Drawn Plot of Disappearance of Oligosaccharides (DP = 7 to DP = 1).
1. Half-life of DP = 7 Material is 120 Seconds
2. All Glucosidic Bonds are Assumed to Break at the Same Rate

TABLE III

CALCULATED DISAPPEARANCE OF INTERMEDIATE CELLODEXTRINS

Time, sec	Concentration, in moles, of various intermediates							
	DP7	DP6	DP5	DP4	DP3	DP2	DP1	Acids
0	1.00							
120	0.499	0.347	0.120	0.027	0.004	0.001	0.000	0.000
240	0.250	0.347	0.241	0.111	0.038	0.011	0.002	
480	0.062	0.173	0.240	0.222	0.154	0.085	0.039	0.023
1200	0.001	0.007	0.023	0.054	0.094	0.130	0.151	0.540
1590		0.001	0.004	0.013	0.030	0.056	0.086	0.810
1890			0.001	0.004	0.011	0.023	0.043	0.918
2190				0.001	0.003	0.009	0.018	0.968
2490					0.001	0.003	0.007	0.989
2760						0.001	0.003	0.996
3030							0.001	0.9985

Linearity of such semilog plots is not necessarily true. Initially in this work we tried to plot the degradation of a cellodextrin by change in the PSA absorbance. Such a curve is not linear (Fig. 8), which may be attributed in part to the inadequacy of this method to determine glucose in the later stages of an alkaline reaction. Linear extrapolation of the first two points gives a value of 10% dextrin remaining in about 40 minutes, or a time of 2400 seconds. The computer calculation (Table III) shows an ideal conversion of 0.989 mole, or about 1% of starting material left.

Two other criteria can be applied. The first is the relatively constant percentage of glucose found for the stopping reaction, ranging from 2.0% at 1 hour to 1.6% at 16 hours (see Table I). The other method is a separation of the

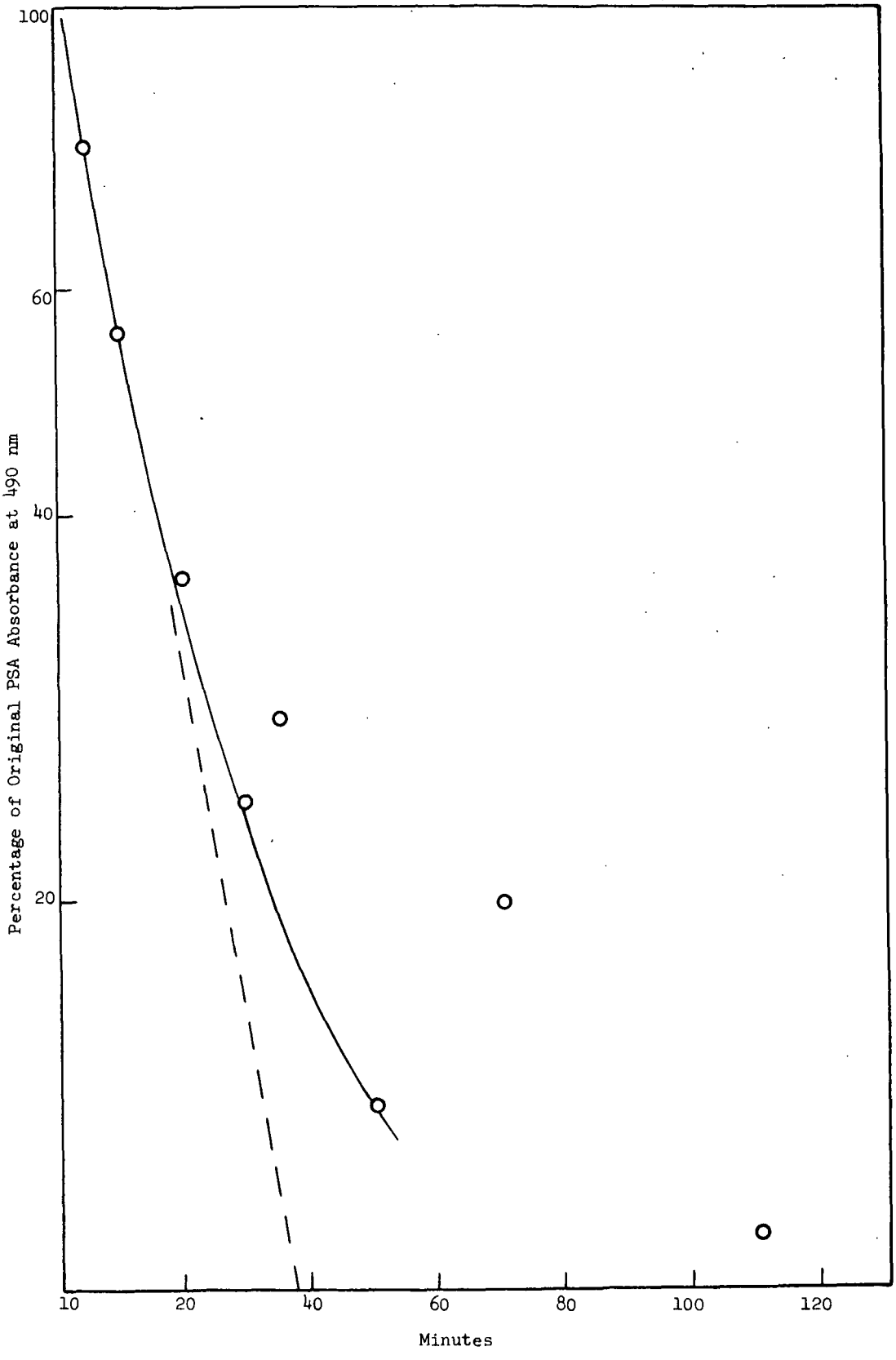

Figure 8. Alkaline Degradation of Cellodextrin by 2N NaOH at 75°C, as Shown by Decrease in PSA Absorbance at 490 nm

acidic products from any neutral products, before hydrolysis, and examination of the latter by gas chromatography. Such a method (see Fig. 9 and 10) does not show any neutral material, presumably undegraded cellodextrins, left in the system.

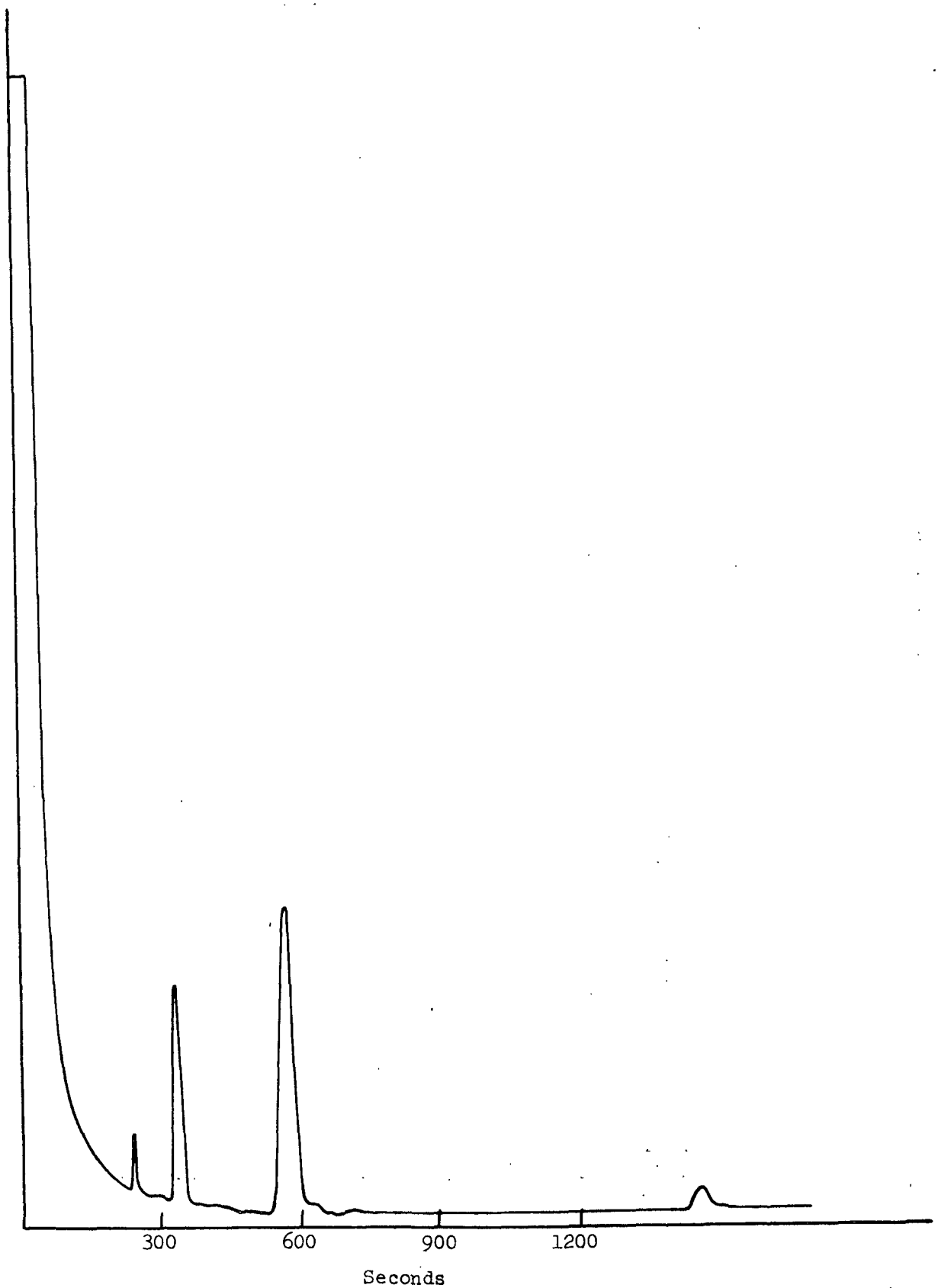

Figure 9. Neutral Degradation Products from Cellodextrin, After MB-3 Treatment. Gas Chromatographic Curve, Trimethylsilyl Derivatives, on OV-17 Column, 6 ft x 1/8 Inch, Programmed 130 to 200°C, at 2°/minute

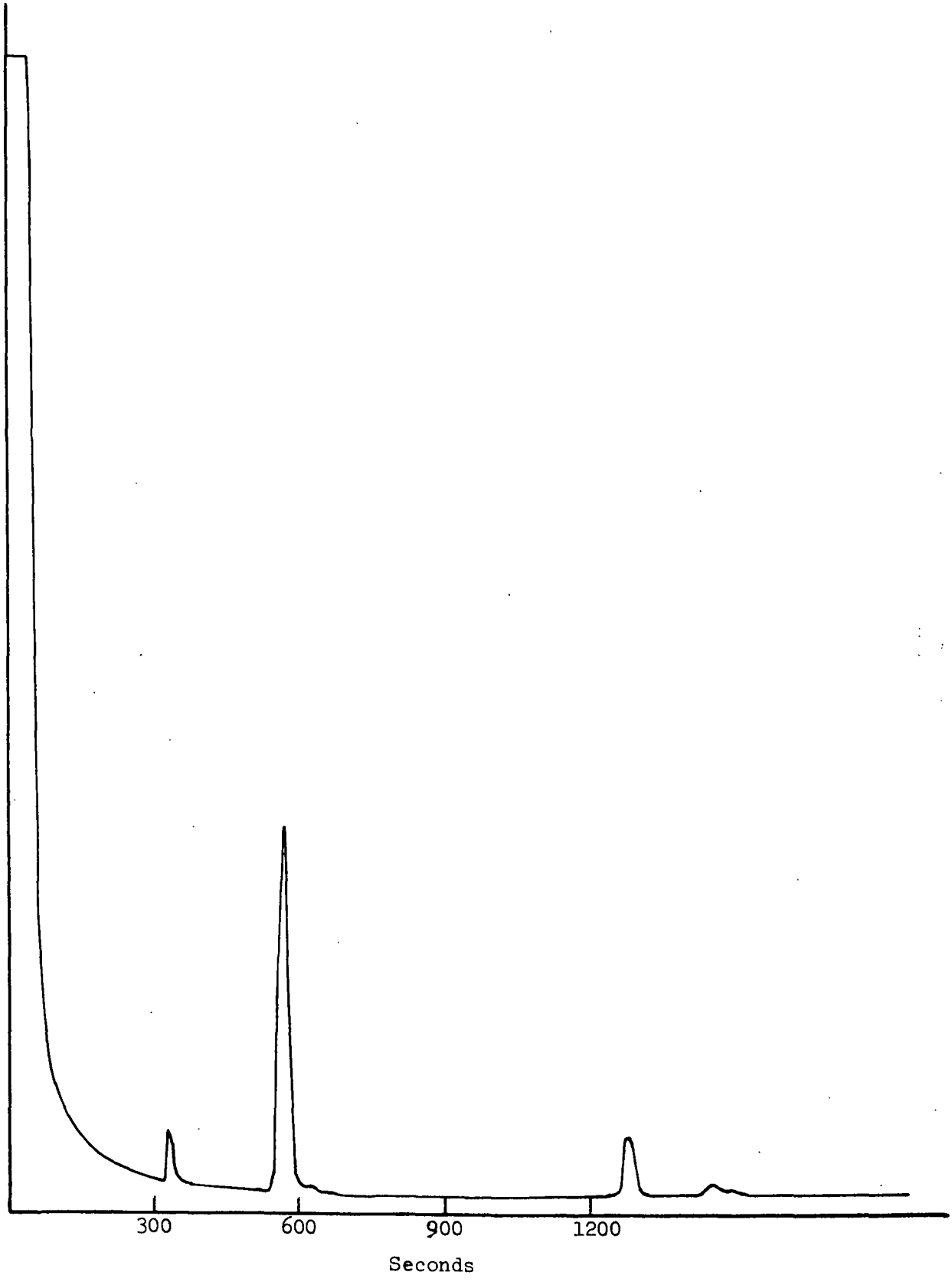

Figure 10. Neutral Cellobiose Degradation Products, After MB-3 Treatment. Gas Chromatographic Curve, OV-17 Column, 6 ft x 1/8 Inch, Programmed 130 to 200°C, at 2°/minute. Trimethylsilyl Derivatives

EXPERIMENTAL PROCEDURES

DETERMINATION OF GLUCOSIDIC BOND IN DEGRADATION PRODUCTS

This procedure was done in three steps, as outlined in Table IV. A sample of cellodextrin (200 mg of average DP 7) was treated with 2N sodium hydroxide (20 ml) at 75°C or 90°C for 1-16 hours. The solution was then cooled, 20 mg of inositol (in the form of a solution) was added as an internal standard, and the solution was treated with Amberlite IR-120 cation-exchange resin to remove sodium ions. The filtrate, Sample A, had a pH of about 3.5. Various aliquots of this, and subsequent fractions, were concentrated to dryness, dried over phosphorus pentoxide and converted to the trimethylsilyl derivatives. The latter were analyzed by gas chromatography on a Varian 1400 chromatograph, and the relative areas of the chromatogram peaks measured with a Varian 485 Digital Printout Integrator. Details of such an integration for glucose and inositol can be seen in Fig. 11.

This Sample A consisted of a mixture of saccharinic acids, mostly the isosaccharinic acid (shown as the lactone by GLC). At the sensitivity run on the gas chromatograph only peaks in the region of C_6 acids or lower were seen. No peaks could be noted at longer retention times, which would be expected for glucosyl-metasaccharinic acids produced by the stopping reaction. Either these acids were of too high molecular weight (lower volatility) to move through the chromatographic column or else their peaks were too small to be detected. The temperature program was run from 100 to 330°C.

The acid peaks of Sample A were completely removed by a mixed bed (Amberlite MB-3) ion-exchange resin. The resulting gas chromatogram showed no peaks at all, just a straight base line.

TABLE IV

TREATMENT OF ALKALINE PRODUCTS TO SHOW STOPPING REACTION

Sample	Mg Original Dextrin	Treatment	GLC Data
A 40 hour dextrin treated with 2N NaOH at 75°C for 15 hours	20	none	Mostly α-isosaccharinic lactone and saccharinic acid
"	40	hydrolysis with N H_2SO_4 at 95°C (this is Sample B)	Same pattern — any glucose peaks are hidden by acid peaks
"	20	MB-3 mixed bed resin	Nothing — all acids are removed
B (hydrolyzed sample with internal standard)	20	none	Area of peaks shows ratio of α-isosaccharinic lactone to standard
"	20	MB-3 mixed bed resin	Area of peaks shows ratio of glucose to standard

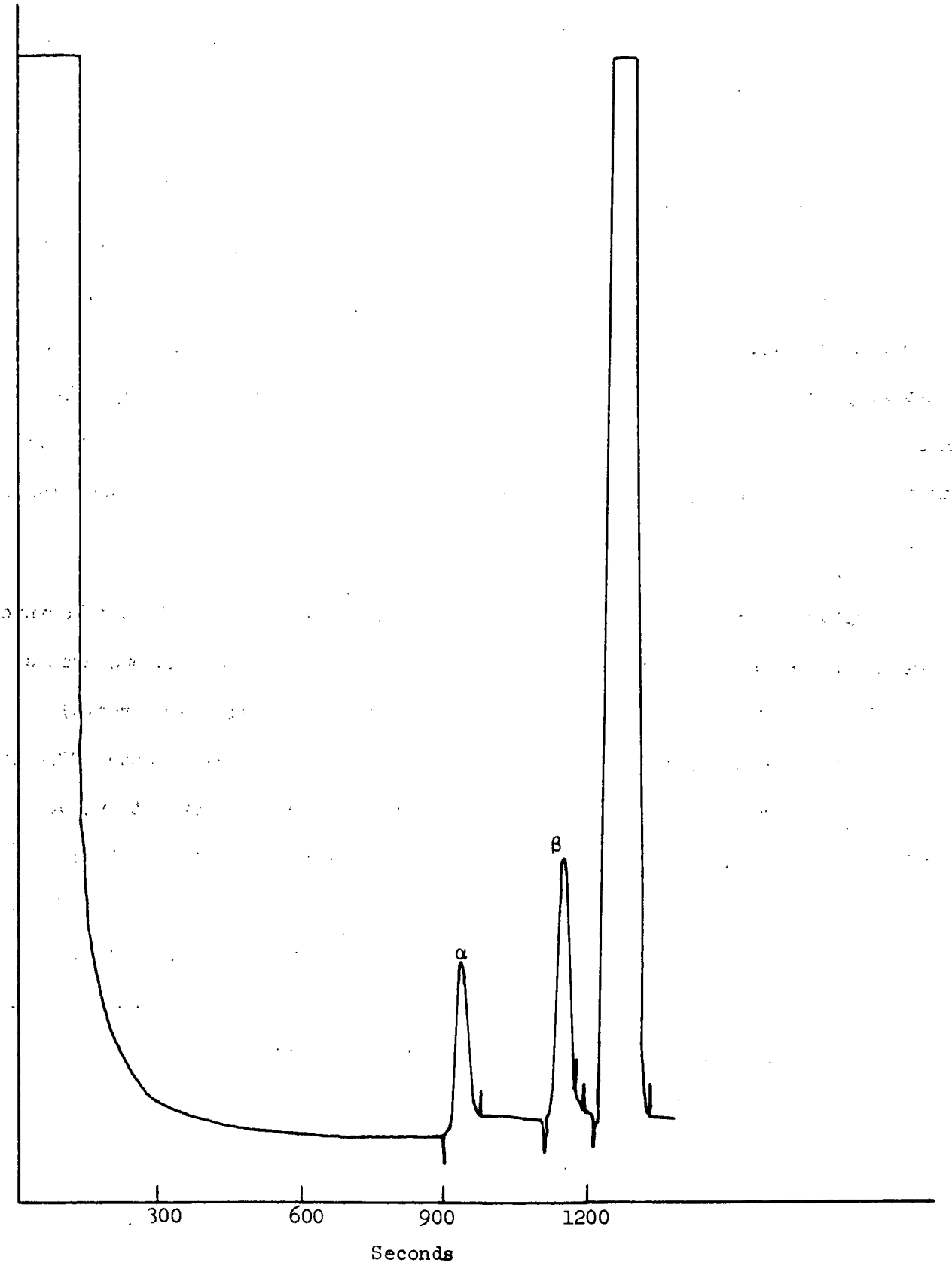

Figure 11. Gas Chromatographic Curve of Trimethylsilyl Derivatives of Glucose and Inositol on OV-17 Column, 6 ft x 1/8 Inch, Programmed 130 to 200°C at 2°/minute (Vertical Lines Represent Integration Marks)

Hydrolysis of a sample of A to produce B (N sulfuric acid for 16 hours at 90°C) was followed by addition of barium hydroxide to pH 5, filtration of the barium sulfate through Celite, and addition of IR-120 resin to the filtrate to remove barium ions.

Sample B was then divided into two portions, and one was treated with MB-3 resin to remove acids and reveal the glucose. The inositol, being neutral, was not removed. The resulting chromatogram gave the relative areas for the glucose and the standard inositol. The other portion was run directly on the gas chromatograph and gave the relative areas of the α-isosaccharinic acid and the standard. From these two sets of data, with the inositol as a common factor, the relative areas of the isoacid, presumably diagnostic of peeling, and of the glucose, diagnostic of stopping, were calculated.

Control experiments were run with mixtures of glucose and isosaccharinic acid lactone; passage of a mixture through a MB-3 resin slowly (about one drop a second for 20 ml of solution, containing 20 mg material, through 20 ml resin) removed the lactone and allowed the glucose to pass through. The glucose effluent did at times give extra peaks, presumably some fructose from isomerization, but predominantly it was unharmed. The pH of the mixed resin ranged from 5 to 7 normally, so there was no alkaline solution to harm the sugar. The effect of the solid basic resins on the sugar solution, however, is unknown. It is felt that this resin is less harmful when mixed with an acidic resin, than when not so mixed. In the latter case the pH of the washings from such a basic resin is about 10 and could harm the sugar solution.

Examples of hydrolyzates, showing saccharinic acids and glucose, are given in Fig. 12-14. Retention times of the trimethylsilyl derivatives are given in Table V.

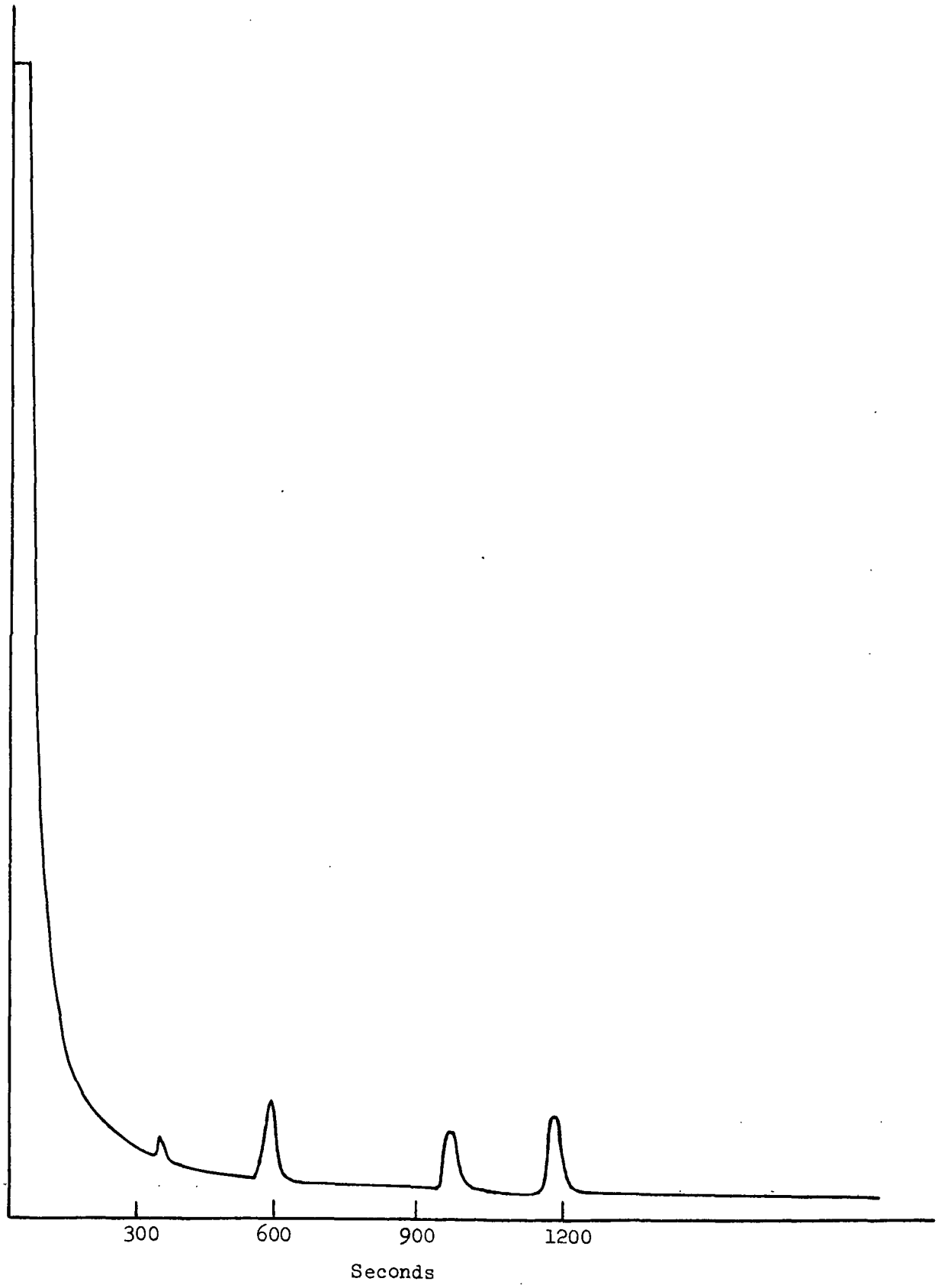

Figure 12. Glucose in Hydrolyzate from Cellodextrin Reaction Products. Gas Chromatographic Curve Run on OV-17 Column, 6 ft x 1/8 Inch, Programmed 130 to 200°C, 2°/minute. Trimethylsilyl Derivatives

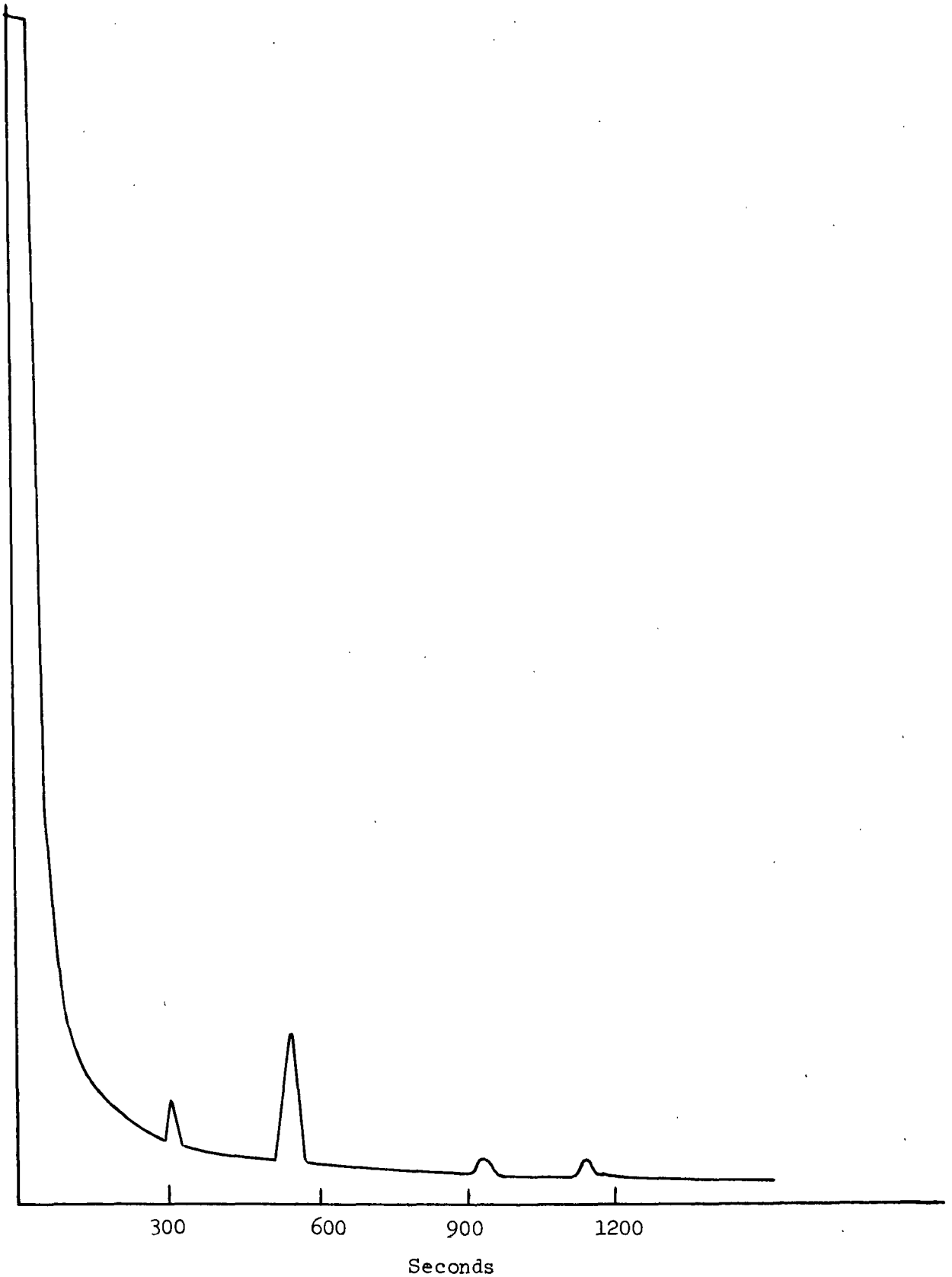

Figure 13. Glucose in Hydrolyzate from Cellobiose Reaction Products. Gas Chromatographic Curve, Trimethylsilyl Derivatives, on OV-17 Column, 6 ft x 1/8 Inch, Programmed 130 to 200°C at 2°/minute

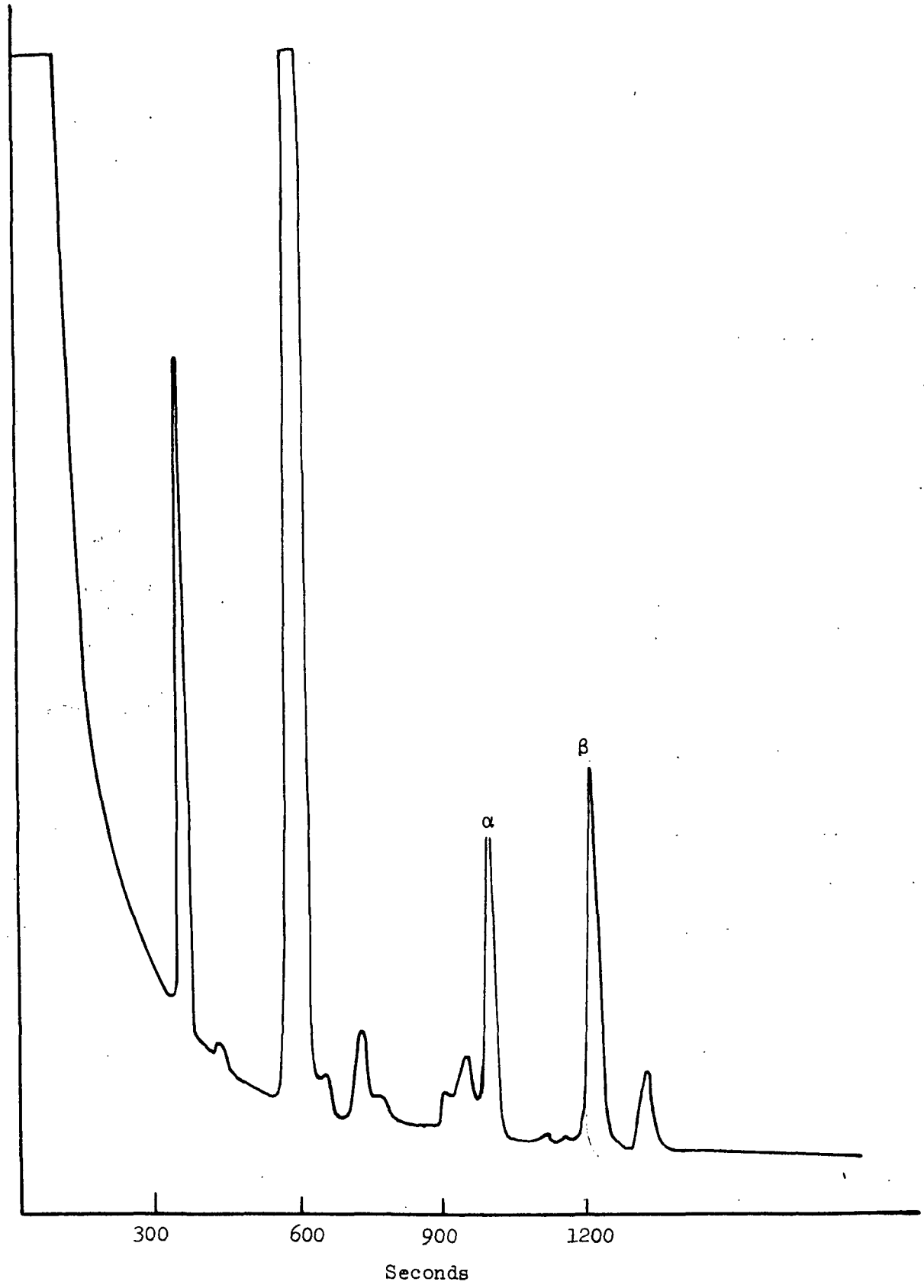

Figure 14. Glucose in Hydrolyzate from Cellobiose Reaction Products. Gas Chromatographic Curve, at High Sensitivity, of Trimethylsilyl Derivatives, OV-17 Column, 6 ft x 1/8 Inch, Programmed 130 to 200°C, at 2°/minute

TABLE V

RETENTION TIMES OF TRIMETHYLSILYL DERIVATIVES

Compound Derivatized	Retention Time on OV-17 Column, sec
α-Glucose	985
β-Glucose	1180
α-Isosaccharinic lactone	690
α-Isosaccharinic acid	890
α-Gluco-metasaccharinic lactone	900
α-Gluco-metasaccharinic acid	940
Inositol	1300

Note — The 6 ft x 1/8-inch column was programmed from 130 to 200°C at 2°/minute, with a nitrogen flow rate of 15 ml/minute. The injection chamber and detector (FID) were maintained at 225°C. Retention times are approximate and often vary by 20 seconds from run to run.

Considerable time was spent in this project on many small aspects of the analysis for glucose. Thus, we found the following:

(1) With a limited wash of 100 ml of water for 20 ml of Amberlite MB-3 resin and a mixture of 20 mg inositol and 2 mg glucose, only 1/3 of the glucose was removed. With a larger wash of 500 ml of water, all of the glucose was removed.

(2) The inositol was added as an internal standard to the alkaline reaction mixture after the latter had been heated and cooled. Incorporation of the inositol before heating gave some loss, apparently some combination of the compound with the reaction mixture. Heating mixtures of inositol and isosaccharinic acid alone in alkali showed no adverse effect.

(3) The best silylating reagent was a two-phase mixture of Tri-Sil Concentrate and dimethylsulfoxide (DMSO). The system was shaken overnight, and then transferred to a smaller vial, from which the upper layer was taken for injection in the gas chromatograph. This is discussed in Report Five, page 19.

(4) Great difficulty occurred with bleeding from the OV-17 column (see a later section dealing with chromatography of the cellodextrins). This was solved to a certain extent by running the normal programming to 200°C, then quickly running the column up to 250°C to remove unwanted peaks, then cooling to between 110 and 130°C until a steady oven temperature of 130°C was obtained. The oven was never cooled below 110°C to avoid the appearance of spurious peaks.

(5) Considerable loss of sensitivity of the flame ionization detector (FID) occurred, due to coating of the electrodes with silica from the trimethylsilyl derivatives. This was solved by cleaning the detector parts frequently with freon or dimethylformamide, and also by use of hot Alconox solution. Sometimes this cleaning was done in an ultrasonic bath. The collector tube was frequently removed and replaced with a clean one. Sometimes injections of freon were made directly on the column.

(6) The various silylation reagents and derivatives were carefully shielded from moisture by storing in a desiccator in a refrigerator, when not in use. When first removed from the refrigerator, they were placed in another desiccator at room temperature to avoid condensation of moisture on the cold containers.

(7) Most solutions were treated with excess IR-120 resin to remove cations (sodium or barium) before concentration to dryness. Addition of IR-120 resin to alkaline solutions until pH 8-9 was obtained, so that sodium salts of

saccharinic acids could be silylated, was often unsuccessful, as a residue of sodium bicarbonate resulted, impeding subsequent derivatization.

PREPARATION OF CELLODEXTRINS AND GAS CHROMATOGRAPHY OF THE TRIMETHYLSILYL DERIVATIVES

Four mixtures of cellodextrins were prepared by the acetolysis of cellulose and subsequent deacetylation of the acetates. The times of acetolysis at 30°C ranged from 16 to 120 hours, and the average DP (degree of polymerization) of the final products ranged from 12 to 2.5. The solubility of the mixtures ranged from complete solubility in water (DP = 2.5) to insolubility in water and solubility in hot 2N sodium hydroxide.

The several dextrin mixtures were analyzed for DP by a sodium hypoiodite method; and for individual components by trimethylsilylation and gas chromatography. By use of a short column and programming to a high temperature, fractions ranging from DP = 1 (glucose) to DP 6 could be readily obtained. Two of the mixtures were also analyzed, as the acetates, by thin-layer chromatography.

EXPERIMENTAL

ACETOLYSIS

The method of Hess and Dziengel (8) was used. A mixture of acetic acid (170 ml), acetic anhydride (170 ml), and sulfuric acid (18 ml) was made up. The first two components were mixed, with cooling in an ice bath, and then the sulfuric acid added slowly. To this chilled mixture, cellulose (45 g) of Whatman powder was added with stirring, and continued cooling. The mixture slowly became a viscous paste, and finally a clear brown liquid after 2-3 hours. It was then placed in an oil bath at 30°C for a given period of time.

The solution was poured slowly into about 2 liters of ice and water and stirred for several hours. The white precipitate was filtered, and resuspended in 1 liter of water. To this suspension was added an aqueous solution of sodium bicarbonate to neutralize any acetic acid present. In some cases considerable foaming occurred. The white precipitate was filtered, washed with water and dried.

In one case, for an acetolysis time of 120 hours, there was a considerable precipitate of cellobiose octaacetate in the acetolysis mixture. This was filtered off and discarded; the clear brown filtrate was added to ice and water as above.

The yields of cellodextrin acetates obtained were about 70-80 grams; the yield from the 120-hour acetolysis was much lower.

DEACETYLATION

The air-dried acetates were dried in vacuo over sodium hydroxide, and then deacetylated by stirring with 0.2N sodium methoxide in methanol for 2-4 hours. Normally, about 1 liter of reagent was used for 70 grams of acetate.

After stirring in stoppered bottles for 2-4 hours, the slurry was filtered and washed with methanol. The original acetates seemed to be insoluble in methanol and the deacetylated dextrins were also insoluble. The latter did not give a positive ester test with ferric hydroxamate reagent (9). The dextrin was stirred with methanol, and a little acetic acid (1-5 ml) added to neutralize any sodium methoxide present.

The yields of cellodextrin were quite low, ranging from 30 grams for the high DP dextrins (starting with 70 grams acetate) to 10-15 grams for the lower DP dextrins. These low yields may have been due to (a) the lower DP of dextrins formed at longer acetolysis times; (b) the use of too much sodium methoxide solution; and (c) the addition of acetic acid to neutralize sodium methoxide. It might be best to use about 5 ml sodium methoxide solution per gram of acetate, and to add very little acetic acid. An explanation of factor (a) above may be that the dextrin acetate first dissolves in the sodium methoxide solution, and then the deacetylated dextrin slowly precipitates out again.

DETERMINATION OF DP

This was done by oxidation of the end group with sodium hypoiodite solution at pH 11. The data for the several cellodextrins are given in Table VI. The oxidations were carried out for varying lengths of time, to ensure complete oxidation. Difficulty was encountered with the 16-hour dextrin, of high average DP, as it was not soluble in the alkaline oxidation reagent.

The average DP of the several mixtures was calculated from the iodimetric data, as shown in Table VII. These calculations were based on data with cellobiose as a known. Since one millimole of this disaccharide (DP = 2) is equivalent to 214 ml of 0.01N thio in the iodimetric system, the weights of the cellodextrin

TABLE VI

IODIMETRIC TITRATION OF CELLODEXTRINS

Sample	Weight of Sample, mg	Time of Oxidation, min	Thio Consumed, ml	Average
16D	40	20	4.65	
			5.00	4.82
	40	40	5.15	
			5.25	5.20
	40	60	5.95	
			5.50	5.72
40D	40	20	8.25	
			8.25	8.25
	40	40	8.15	
			8.15	8.15
	40	60	8.55	
			8.45	8.50
72D	30	20	8.65	
			8.55	8.60
	30	40	8.95	
			8.95	8.95
	30	60	8.85	
			8.95	8.90
120D	40	20	19.40	
			19.50	19.45
	40	30	19.70	
			19.95	19.82
	40	50	19.80	
			26.35	20.08
Cellobiose	20	20	12.35	
			--	12.35
	20	30	12.65	
			12.65	12.65
	20	60	11.95	
			13.05	12.50

Note — 16 D, etc., refer to the hours of acetolysis required to produce this cellodextrin.

mixtures, equivalent to this amount of thio, were calculated. From these weights, the average weight of a millimole, the average DP values were obtained. The trend is very good, the average DP decreasing with increasing time of acetolysis.

TABLE VII

CHARACTERIZATION OF CELLODEXTRINS

Time of Acetolysis, hours	Ml 0.01N thio per 10 mg	Ml 0.01N thio per 342 mg G_2	Mg Sample per 214 ml thio	DP
--	--	214	342	2
16	1.3125	--	1630	10-11
40	2.075	--	1031	6-7
72	2.93	--	730	4-5
120	4.99	--	434	2-3

Note — One millimole dextrin = 162n + 18 mg where n = DP of the dextrin.

The experimental procedure, that of Willstatter and Schudel (10), consists of adding 10 ml of 0.035N KIO_3 to the cellodextrin solution, then 500 mg KI and 5 ml of 1N H_2SO_4; the liberated iodine is converted to sodium hypoiodite by the addition of 0.5N NaOH (usually 10-12 ml) to a pH of 11 to 12. The solution is left in the dark for 20 to 60 minutes, then acidified and the excess iodine titrated with 0.01N thiosulfate.

The addition of the alkali to the iodine solution to form hypoiodite is done in one stage, with a graduated pipet; toward the end of the addition the iodine disappears and the solution turns light yellow. It is best to check the pH in all cases, to insure that it is between 11 and 12.

The sugar must be added to the iodine system before the alkali is added; if the reverse addition is done, low values are often obtained. This is because

the alkaline hypoiodite system rapidly disproportionates to iodate and iodide; this second system will not oxidize cellodextrins, and so a low final thio titration is obtained.

GAS CHROMATOGRAPHY

Samples 1-3 mg cellodextrin were weighed into a cone-shaped 1-ml Reacti-vial, and dried in vacuo over phosphorus pentoxide for 2-16 hours. Then 0.3 ml each of dimethyl sulfoxide and Tri-Sil Concentrate were added, the vial capped with a septum cap (lined with teflon) and shaken for 4-16 hours. Samples (1-3 microliters) of the trimethylsilylated samples were injected onto an OV-17 column (2 feet x 1/8 inch) and programmed from 160 to 330°C at 6°/minute, after a holding period of ten minutes at 160°C. A sensitivity of 64X and 10^{-11} was used to give good peaks on the recorder.

The glucose came through very rapidly (2 peaks); the cellobiose peaks (2) came at 17 minutes (200°C) and the DP 3, 4, and 5 peaks at 25, 30, and 33 minutes. With some difficulty the DP 6 and DP 7 peaks could be obtained (see Fig. 15).

Both the 16 and 40 hour dextrins showed peaks up to DP 7, with emphasis on the latter peaks. In contrast, the 120-hour dextrin has a major peak at DP 3, with large amounts of DP 2 also.

The above injections of samples were made directly onto the column in the injection chamber. The latter was maintained at 300°C and the detector at 325°C.

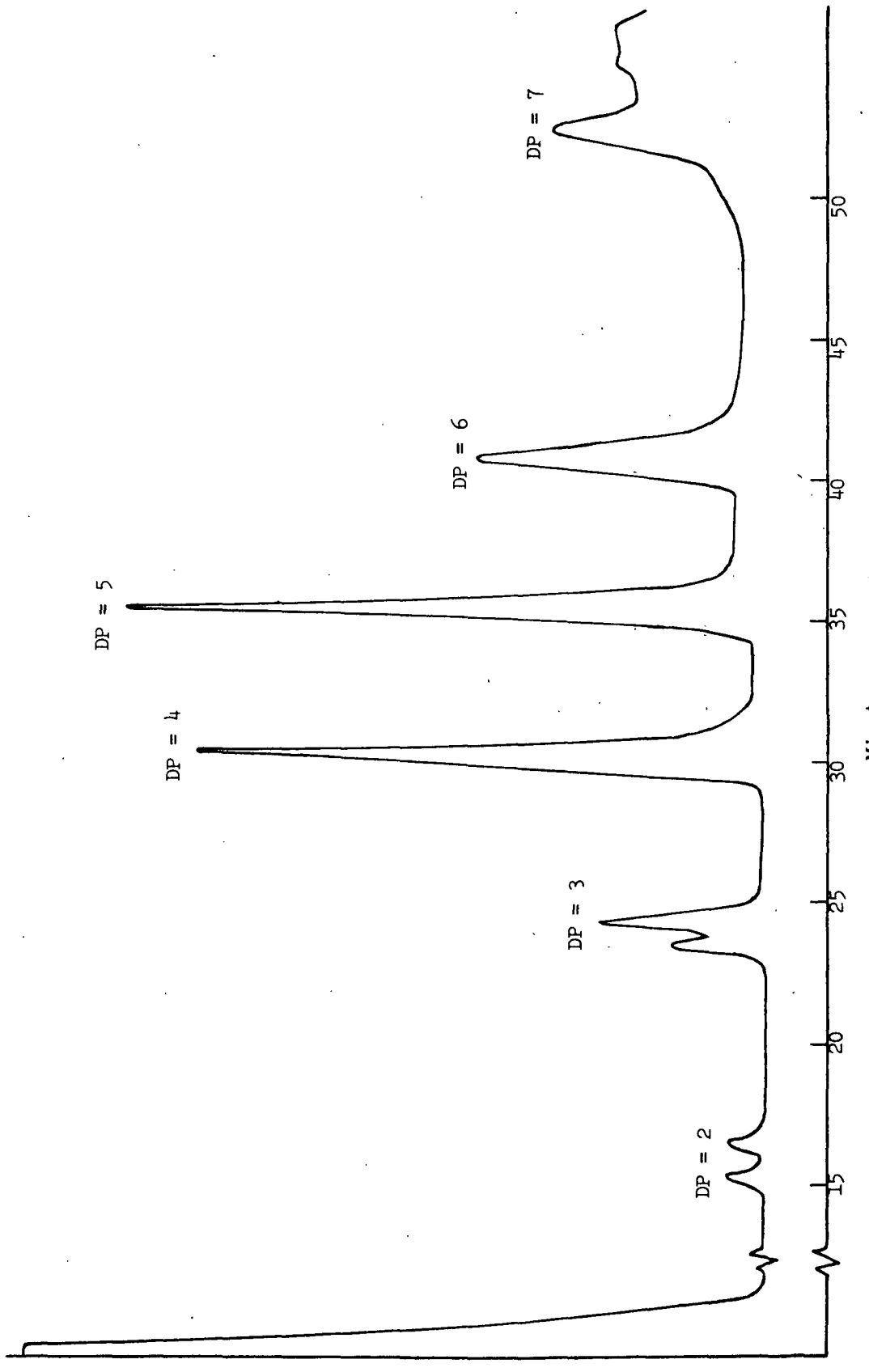

Figure 15. Gas Chromatographic Curve of Trimethylsilylated Cellodextrin, Run on OV-17 Column, 2 ft x 1/8 Inch, Programmed 100 to 330°C, at 6°/minute

SPURIOUS PEAKS FOR ON-COLUMN INJECTION ON OV-17 COLUMN

This chromatographic column is normally operated at temperatures ranging from 50 to 370°C and with injector temperatures up to 300°C and detector temperatures up to 325°C. This is shown in Fig. 16A. Ideally, there should be a low bleeding of material from the column, in a continuous manner, and this is shown in Fig. 16B. This continuous bleed will occur when the injector temperature is at a low temperature (below 200°C) and a relatively low sensitivity (10^{-11}, 512 attenuation). Above 300°C oven temperature there is a sharp rise in column base line.

At higher injector temperatures problems occur when the column is cooled down before temperature programming. Normally, the column is cooled to 50°C (open oven door) and then warmed up to 100°C (closed door) before programming during a run (100-370°C). However, with the hot injector zone and cold column, there is apparently an initial condensation of material bled from the column; this occurs in the first part of the oven portion of the column (Fig. 16C). When the column is warmed up, in a temperature program, this material moves gradually through the column and emerges at the detector as a series of peaks (Fig. 16D). These peaks will come through up to a temperature of 250°C and cause problems in analysis of trimethylsilylated materials.

The peaks can be prevented by cooling the column down to a temperature of 150°C and then running a program from 160 to 370°C. If the column is cooled below 150°C, the peaks will appear on subsequent programming.

In the analysis of cellodextrins, as the trimethylsilylated derivatives, the injector temperature must be maintained at 300°C; at lower temperatures the higher DP dextrins will not move from the injection chamber. Cellobiose will not

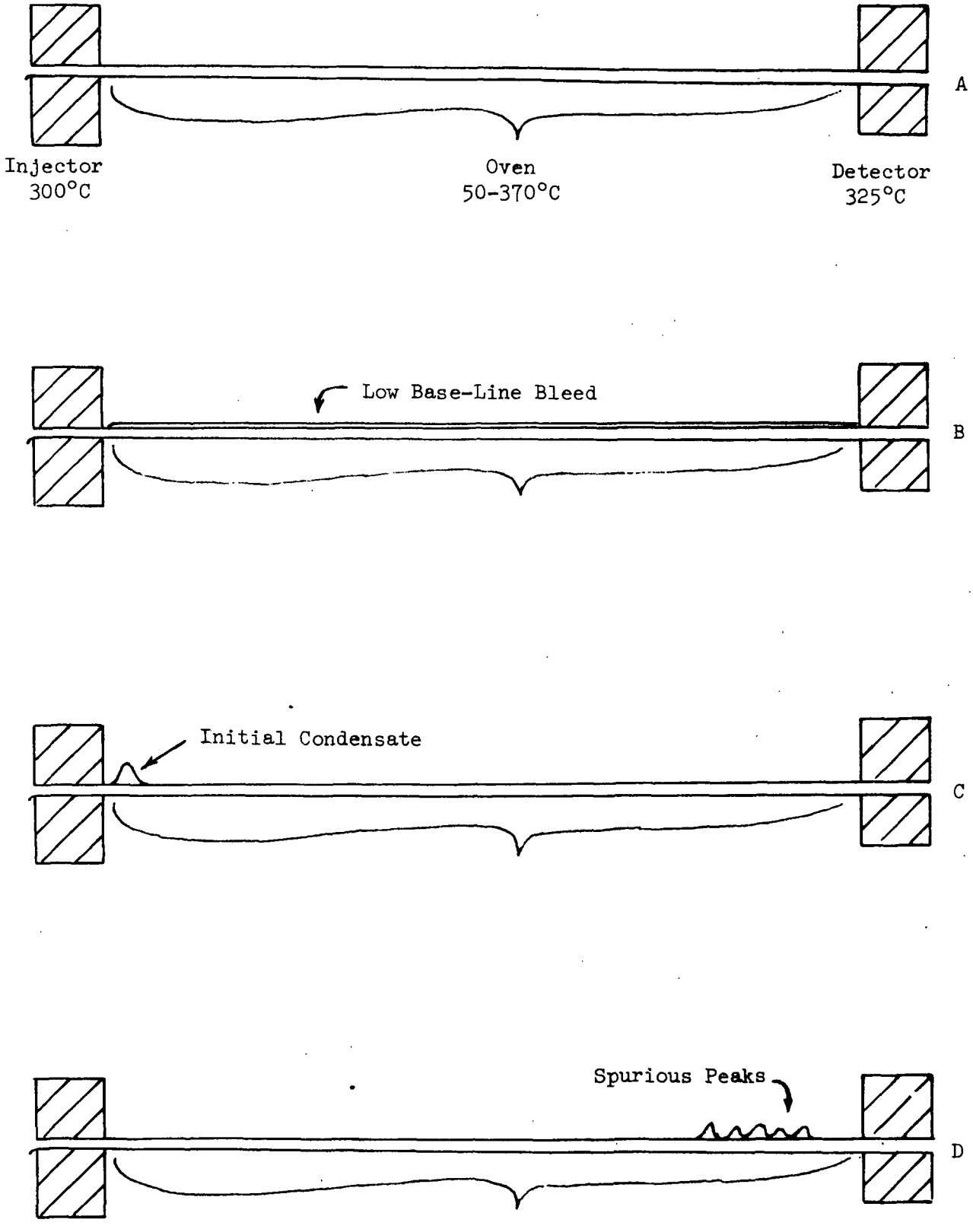

Figure 16. Problem of Hot Injector and Cold Column (the First 3 Inches of the Column are in the Injection Chamber)

move from the injection chamber below 200°C. So we cannot solve the problem by use of a lower injection temperature. The samples vaporize on the column in the injector chamber, and then move along the column to the detector region as the temperature of the column is increased.

Another possible solution (suggested by the Supelco Company, suppliers of chromatographic columns) is to use a cold injection chamber and inject directly on the column within the oven. For this a syringe with a long needle (5 inches) is needed; two such needles have been ordered from the Hamilton Company. The sample will be deposited where the "initial condensate" is formed in Fig. 16C, and will gradually move through the column as the temperature is raised. With this system there should be no spurious peaks, and just a low base-line bleed as shown in Fig. 16B.

Finally, it should be mentioned that the injection port is a large piece of metal and its temperature cannot be programmed readily, as can the oven containing the column. It requires at least 30 minutes to heat this chamber up to 300°C and a much longer time to cool it down.

THIN-LAYER CHROMATOGRAPHY OF CELLODEXTRIN ACETATES

There is a limitation to the resolution of cellodextrins by GLC; dextrins with DP greater than 7 cannot be shown as peaks on the recorder. In contrast, thin-layer chromatography will show many more peaks, when the acetates are run with a benzene-ethanol (10:1) mixture. Such separations were run with the 72 and 120-hour dextrins. The mixtures were spotted on glass plates coated with silica gel, developed with the above mixture and the dried plates sprayed with 50% sulfuric acid and then heated below 300°C.

The 120-hour dextrin showed primarily cellobiose and cellotriose, as the α-acetates. There were minor traces of the β-acetates, also traces of glucose. In contrast, the 72-hour dextrin showed primarily fractions from DP 2 to DP 5, and traces of glucose and of DP 6 and 7. Very faint traces were shown above DP 7. The 120-hour dextrin, however, showed a faint spot for the DP 4 fraction and a very weak spot for DP 5.

In summary, the 120-hour dextrin was composed primarily of DP 2 and DP 3 fractions, and the 72-hour dextrin primarily of DP 2 to DP 5 fractions. It should be recalled that yields after deacetylation are not quantitative and, therefore, part of the acetate fractions were lost, probably those of lower DP.

THE INADEQUACY OF THE PSA METHOD FOR DETERMINING GLUCOSIDIC BONDS IN ALKALINE DEGRADATIONS

The PSA method (phenol-sulfuric acid) has been used to measure small concentrations of reducing sugars, disaccharides and glucosides; the color developed is measured at 490 nm. Glucosidic bonds in disaccharides and glucosides are hydrolyzed by the hot acidic reagent so that color is then developed from the glucose liberated. This method was used successfully by Lindberg, et al. (1) to follow the disappearance of disaccharides in alkaline media. They also used this method to determine a small amount of stopping reaction; the "residual absorbance" formed by alkaline solutions, after long reaction times, with the PSA reagent, was attributed to alkali-stable glucosidic bonds, which were presumably present as glucosyl-metasaccharinic acids.

In our studies we have used this method and found some "residual absorbance," but not as much as that reported by Lindberg, et al. (1). However, this method did seem to show a certain amount of stopping reaction, accompanying the peeling of cellobiose in alkaline solutions. Unfortunately, we have also found two adverse facts that discredit this method, as a means of studying the stopping reaction.

First, glucose, which has no glucosidic bonds, also gives a "residual absorbance" at 490 nm, after treatment with alkali (see Table VIII). If all the glucose were destroyed by the alkali, a logical assumption, then the PSA method should give no color in the 490 nm region as a measure of glucose.

Secondly, the absorbance curve for fresh glucose or cellobiose ("original absorbance") produced with the PSA reagent differs greatly from the curve for "residual absorbance," obtained after alkaline treatment of the sugars.

The "original" absorption curves for the untreated sugars (Fig. 17) show a single maximum at 490 nm. In contrast, the "residual" absorption curves for the sugars, after treatment with alkali, show two maxima, a small one at 490 nm and a large one at 415 nm. So apparently the "residual absorbance" cannot be attributed to glucose, derived from a glucosidic bond, but must represent one or more artifacts, that also give color with the PSA reagent.(Fig. 18).

TABLE VIII

RESIDUAL ABSORBANCES SHOWN BY ALKALINE GLUCOSE SOLUTIONS

Time of Heating at 75°C (for glucose in 0.2N NaOH)	Absorbance at 490 nm
Zero hours	1.300
72 Hours	0.163

In confirmation of this, the artifact derived from glucose is acidic, similar to that derived from cellobiose (see Report Five, page 8). It is retained on MB-3 resin, and most of it can be removed by washing with alkali. Thus, of the material absorbing at 490 nm, in the PSA test, 80% was retained by the resin, and then recovered. Of that absorbing at 415 nm, 62% was retained by the resin, and then recovered. The data are given in Table IX.

One might say at first thought that the residual absorbance is composed of two factors, a peak at 490 nm derived from glucose, and a peak at 415 nm derived from an unknown artifact. But this thought is negated by the fact that the 490 nm peak is also found when glucose is treated with alkali; here all the glucose must be destroyed and so the 490 nm peak must also be attributed to an artifact.

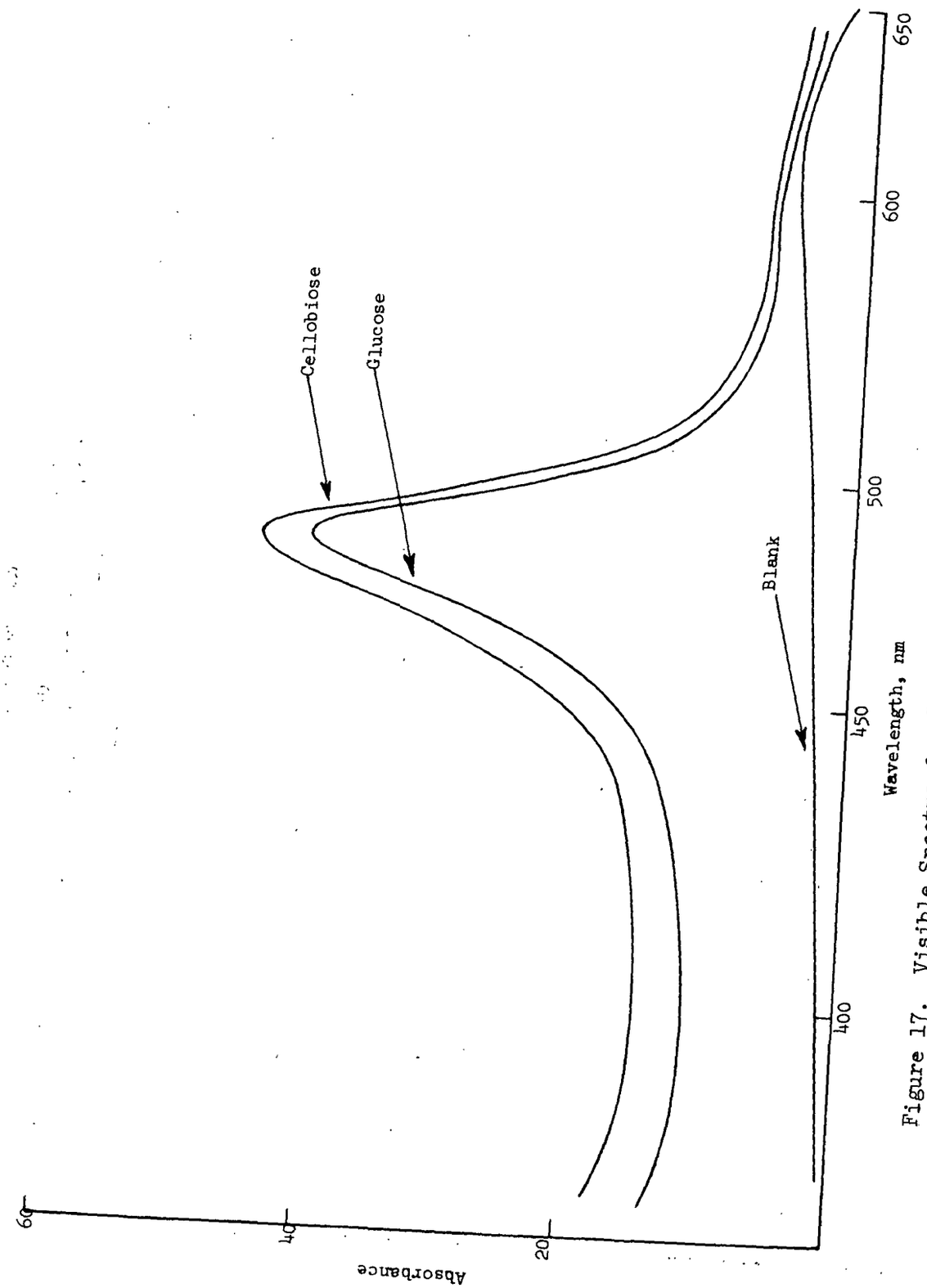

Figure 17. Visible Spectra for Glucose and Cellobiose Color Developed by PSA Reagent

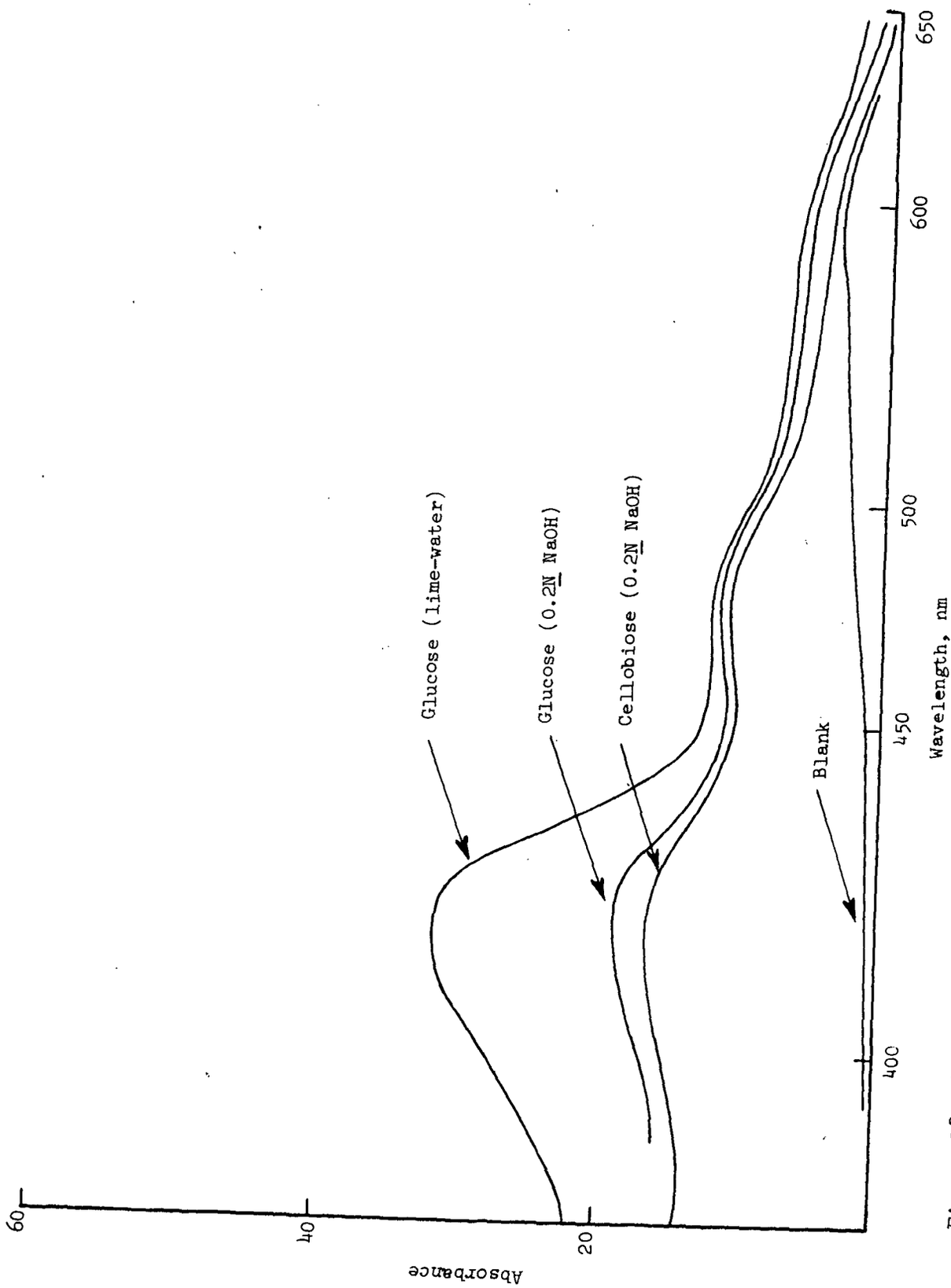

Figure 18. Visible Spectra for Reaction Systems of Glucose and Cellobiose After (a) Treatment with NaOH or Lime-Water; (b) Color Developed with PSA Reagent

TABLE IX

ACIDIC MATERIAL, DERIVED FROM GLUCOSE, RETAINED AND RECOVERED FROM MB-3 RESIN

Sample	Absorbance, after PSA test, at 415 nm	490 nm
Original sample[a]	0.498	0.163
Effluent from MB-3 column		
first 100 ml	0.016	0.024
second 100 ml	--	0.004
Alkaline effluent	0.309	0.131

[a] This was obtained by heating glucose 72 hours in 0.2N NaOH at 75°C.

The similarity of the "residual absorbance" of glucose and cellobiose can be attributed to glucose being an intermediate in the peeling of cellobiose; it is then degraded in a manner similar to that for solutions containing only glucose.

Thus, it seems necessary to use another method than the PSA method to determine the presence of glucosidic bonds, formed by the stopping reaction in the alkaline degradation of oligosaccharides. The original data cited by Lindberg, et al. (1) for the stopping reaction seems to be invalid.

A final example of the inadequacy of this method has been cited earlier (Fig. 8). A cellodextrin was treated with 2N NaOH at 75°C for periods of time up to 45 minutes. The disappearance of the dextrin was followed by the PSA method. It can be seen that the semilog plot is not linear. A straight-line extrapolation of the first two points shows that most of the dextrin is gone in 35 minutes; this agrees with other experiments (gas chromatography and hydrolysis) which show very little dextrin left at a 1-hour period of heating. So it appears that artifacts are appearing which give spurious results.

EXPERIMENTAL DETAILS

A solution of 50 mg anhydrous glucose in 50 ml of freshly boiled 0.2N NaOH was heated 72 hours under nitrogen at 75°C. Portions of this solution, and of a similar unheated solution were treated with the PSA reagent (1 ml of solution, 1 ml of 5% phenol solution, and 5 ml of concentrated sulfuric acid) and absorbances determined at 415 nm and at 490 nm. Also absorption curves were run for the two above solutions (after PSA treatment), for a solution similarly treated with 0.05N lime water and for a solution of untreated cellobiose (see Fig. 17 and 18). All solutions were of the same concentration, of 1 mg original sugar per ml solution. The data are given in Tables VIII and IX.

A column of 25 ml MB-3 resin was washed with 500 ml water. A 25-ml aliquot of the glucose solution, after treatment with 0.2N NaOH for 72 hours at 75°C, was passed through the column and two 100-ml volumes of effluent collected.

The column was then treated with 30 ml of N NaOH solution and washed with water, to a total volume of 400 ml washings. The first 100 ml were strongly alkaline, the last washings were neutral (pH 7). The combined washings were treated with IR-120 resin to remove alkali; the filtrate, with a pH of about 3, was concentrated to 25-ml volume and an aliquot (1 ml) treated with the PSA reagent. Absorbances of the several solutions were determined at 415 nm and at 490 nm. The data are given in Table IX.

GLOSSARY OF TERMS USED

DMSO	Dimethyl Sulfoxide
DMS	Cellodextrin-metasaccharinic acids; DP > 2
DP	Degree of polymerization
FID	Flame Ionization Detector
GLC	Gas liquid chromatography, often called gas chromatography
GMS	Glucosyl-metasaccharinic acid (a C_{12} acid, which should not be confused with glucometasaccharinic acid, a C_6 acid); DP = 2
PSA	Phenol-sulfuric Acid

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LITERATURE CITED

1. Lindberg, B., Theander, O. and Uddegard, J., Svensk Papperstid. 69:360 (1966).

2. Lai, Y., and Sarkanen, K. V., Cellulose Chem. Technol. 1:517(1967); J. Polymer Sci. Part C, (28):15-26(1969).

3. Rowell, R. M., Somers, P. J., Barker, S. A. and Stacey, M., Carbohyd. Res. 11:17(1969); Rowell, R. M., Pulp Paper Mag. Can. 72:T235(1971).

4. Alfredsson, B., Gedda, L., and Samuelson, O., Svensk Papperstid. 64:694 (1961).

5. Nef, J. U., Ann. 376:1(1910).

6. Green, J. W., J. Am. Chem. Soc. 78:1894(1956); Ishizu, A., Lindberg, B. and Theander, O., Acta Chem. Scand. 21:424(1967).

7. Frost, A. A., and Pearson, R. G. Kinetics and mechanism. p. 166. New York, John Wiley and Sons, 1961. The Computer program made from the equations in this chapter will be available to any cooperator upon request.

8. Hess, K., and Dziengel, K., Ber. 68:1594(1935).

9. Cheronis, N. D., and Entrikin, J. B. Semimicro qualitative organic analysis. p. 229. New York, Interscience, 1961.

10. Willstatter, R., and Schudel, G., Ber. 51:780(1918).

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